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GROWTH OF THE MAIZE ROOT TIP

by

Gabriel Baldovinos-de la Pena

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TABLE OF CONTENTS

INTRODUCTION

The present thesis is a continuation of work begun in 1946 and reported in part in 1947 as an unpublished master's thesis with the title, Growth of Corn Roots (2). That thesis discussed the processes of cell division and cell enlargement in the maize root tip. The data presented showed that the cells of the first millimeter behind the root cap are nearly square in longitudinal section, non-vacuolated, and densely protoplasmic. In the second millimeter the cells were more elongated and showed vacuolation, especially toward the proximal end of the section. Cell division was limited to these two sections, being at the rate of about once in 24 hours at 15° C. and nearly twice as fast at 25° C. The second section, which showed rather rapid cell elongation, had a specific cell division rate somewhat above that of the first millimeter. No cell division was observed behind the third quarter of the second section. Cell enlargement was rapid in the third section, particularly at the proximal end, slower in the fourth, and generally absent from the fifth and sixth sections.

The present study has added to this picture the changing chemical composition of the three growth zones, including studies of metabolites, protoplasmic and cell wall materials; respiratory rates and reactions, and changes in hydration with growth under various conditions. Tissue culture techniques have been used freely to accentuate zonal reactions, and a special study of the utilization of glucose-1-phosphate has been included.

REVIEW OF LITERATURE

Growth of corn roots (2), of which this thesis is a sequel, contains 39 references, mostly on cell division and enlargement. To conserve space only a few of these references are repeated here. The earlier thesis should be consulted for the others.

The movement of food and minerals varies within radicles, depending upon the special type of tissue involved. Esau (14) pointed out a distinct differentiation of alternate bands of phloem and xylem at 500 mu. above the radicle cap base in graminaceous roots. At the same level, cortical cells still remained thin-walled and small in size. In the region of rapid thickening of secondary cortical cell walls the vascular bundles were more stratified with larger contents of cellulose, lignin, pectins, and other organic and inorganic products. Permeability differences between cells in pre-division and those in active division were investigated by Stern (46) who grew pollen mother cells and microspores of Trillium in sucrose and glucose media. In pre-meiosis the pollen mother cells were found to be freely permeable to sucrose, but by the time active division started a drop in

permeability was estimated. Some time after completion of meiosis the microspores were found to be impermeable to sucrose and glucose; with the onset of active mitosis their permeability increased again showing a maximum at mitotic prophase.

The *in vitro* inhibition of absorption offers valuable possibilities for studying differential permeabilities of the plasma membrane to specific compounds. Chang and Loomis (6) showed direct inhibition of water and mineral absorption by wheat, rice and corn seedlings grown in nutrient solution with increased CO_2 concentration around the roots, and they thought that high CO_2 led to the formation in the protein component of the plasma membrane of specific, although weakly bonded hydrogen bridges on the more basic amino groups above the alpha carbon, or possibly on cyclic nitrogen atoms. The cross linkages between adjoining protein molecules could rapidly decrease their dispersion and increase the viscosity of the plasma. An inhibitor which prevents the active transport of an essential compound across the membrane might not necessarily interfere with its utilization once inside. Mitchell and Houlahan (34) found that in the *Neurospora* mutant requiring vitamin B_2 for growth, added amounts of lumichrome and

lumiflavin inhibited the utilization of that vitamin. Neither lumiflavin nor lumichrome inhibited growth of the wild type organism which was able to synthesize its own B_2. It may be then that lumichrome and lumiflavin blocked the transference of B_2 across the membrane. When the vitamin was formed inside the cell, the inhibitors were not effective in interfering with its function.

The inhibition of translocation by uranium has been explained as due to the combination of this metal with protein layers of the cytoplasm membrane, thus bringing about changes in permeability and affecting the passage of certain substrates. Barron et al. (3) studied the effects of uranyl nitrate on yeast cells and yeast extracts, finding that glucose fermentation by yeast which had been inhibited by uranium was completely released on addition of phosphate. Phosphate, however, did not release inhibition of fermentation in cell free yeast juice. It was concluded therefore that inhibition was due not to combinations of the metal with enzyme systems within the cell, but rather to the adsorption of the metal in the cytoplasmic membrane making it impermeable to glucose.

The transport of simple sugars to metabolizing cells has been substantiated for a range of living systems. Changes

in the concentration of reducing sugars leads to a wide variety of effects, for these compounds provide the energy and basic raw materials for active synthesis. Shifts in the starch-sugar balance have been visualized as auxin induced stimulations of the cell diastase activity (15). An investigation of the action of indoleacetic acid in a diastase charcoal system was made by Smith et al. (45), and no convincing evidence was found that auxin either inhibited free diastase directly or markedly altered the absorption equilibrium of diastase on charcoal.

Arreguin-Lozano and Bonner (1), comparing the hydrolytic breakdown of starch under the influence of amylase and the phosphorolytic breakdown via phosphorylase, concluded that starch metabolism in potato tissues occurred under the influence of phosphorylase, as potato tubers showed only weak amylase activity, and they estimated powerful phosphorylase activity. At either low or high temperatures phosphorylase concentration remained constant. Since at high temperatures no starch breakdown took place, they suggested that phosphorylase activity was probably regulated by a specific inhibitor formed at high temperatures and absent or present in lower concentrations at low temperatures, the formation, degradation and nature of which still remained to be explained.

A study of the changes in carbohydrate distribution occurring as a result of the growth of excised tomato root tips supplied with sucrose, suggested to Dorner and Street (13) the existence of a mechanism involving the breakdown of a sucrose molecule into its hexose units coincident with the absorption of one dextrose unit for each two molecules of sucrose degraded. The possibility that sugar absorption in vitro by the epidermal cells involved phosphorylation at the plasma membrane and dephosphorylation with liberation of free sugar at the cytoplasm was tentatively advanced, suggesting that sugar movement through the plasma membrane occurs as hexose phosphates.

As a result of studies on nitrogen metabolism, several possible pathways have been proposed and still others remain to be worked out. Great emphasis has been given to the mechanisms by which amino acids are synthesized or deaminated to yield the corresponding alpha keto acids which can be further oxidized to CO_2 and water. McVicar and Burris (33) detected a very rapid absorption of isotopic ammonium nitrogen by roots of tomato plants grown in nutrient solution. They found also that the labeled material spread rapidly over the entire plant,

with more movement to the actively growing regions, but did not determine whether there was a direct translocation of the ammonium ion to the rest of the plant organs or whether there was an initial conversion of the ammonium ion to organic nitrogenous compounds with subsequent transfer.

Nitrate nitrogen absorption has been coupled to carbohydrate oxidation because the reduction of nitrates to ammonia, considered to be an initial step in nitrogen assimilation, is an endergonic reaction. The existence of one mechanism has been postulated by Nance (35) in which reductions taking place under aerobic conditions were ascribed to a coupling of the nitrate reducing mechanisms with mechanisms generally considered part of the anaerobic phase of carbohydrate breakdown. He found in excised wheat roots grown *in vitro* that oxygen inhibited assimilation of previously absorbed nitrate, and that nitrate increased in the absence of oxygen, suggesting that nitrate assimilation occurred under conditions that favored aerobic fermentation, and that systems concerned with anaerobic degradation of carbohydrates used nitrates as terminal oxidants.

Culpepper and Moon (12), studying the distribution of food materials during the development of asparagus stems and

their relations with growth, observed that total sugars were most concentrated at the stem base, decreasing rapidly toward the growth zone, and, conversely, that nitrogen was lowest at the basal zone, increasing rapidly near the tip. Loomis (27) found that root formation in Melilotus alba did not take place when roots were decapitated to remove cotyledonary buds. Carbohydrate reserves were high, and probably higher than the percentage sugars estimated since there were changes in the starch sugar balance prior to killing. The soluble organic nitrogen also was inversely correlated with root formation.

Anatomical and metabolic characteristics of root meristems pertinent to their use in investigations involving measurements of gas exchange have been correlated with estimations of the rate of cell division and quantity of protoplasm per unit volume of tissue accompanying the differentiation and maturation of cells.

Lund and Kenyon (29) estimated high reduction rates of methylene blue at the root tip of Allium cepa, decreasing progressively as the measurements were made in zones approaching the basal region, with a minimum about 30 mm. from the root tip. They thought that large utilization of oxygen with the corresponding CO_2 evolution per root length was indicative of highly electropositive zones,

and that at any root level the specific quantitative relationship in flux equilibrium between oxygen consumption and electromotive forces was maintained by local enzymatic oxidation-reduction systems. Gregory and Woodfard (17), making direct determinations of the oxygen consumption in the first five apical centimeters of Vicia faba roots, estimated that the first 10 mm. segment was characterized by the largest rate of oxygen consumption, despite the fact that both volume and surface were the smallest.

Machlis (31) studied respiratory quotients of unbranched barley roots cut into 10 mm. segments and grown in dilute nutrient solution, and calculated for the first 10 mm. segment a respiratory quotient (R.Q.) around 0.94; for the succeeding segments the R. Q. became essentially equal and close to 1.00, suggesting a distinct gradient in respiratory activity from the apical root region to the basal, and that the main course of respiration beyond the 10th mm. could be qualitatively the same.

Recent developments (9) in the study of respiratory mechanisms have yielded several pieces of evidence substantiating the proposition that cell respiration is not carried out by a single continuous system, but rather that each oxido-

reduction reaction within a given respiratory chain seems to have a specific course of activity. An example of this type of evidence is the data on the cyanide sensitivity of respiration, which in turn is attributable to external factors such as concentration of molecular oxygen and of metabolite substrate. Thus, Machlis (30) found that cyanide inhibited two-thirds of the oxygen consumption in excised barley roots and Henderson and Stauffer (20) found that in excised tomato roots a 5×10^{-3} M cyanide concentration inhibited 79 percent of the normal oxygen uptake, and 1×10^{-2} M concentration almost stopped oxygen absorption.

The participation of sulphydryl groups in respiration and growth has been studied by experiments involving a direct chemical combination of terminal -SH groups with specific inhibitors. Changes in specific rates of gas exchange could be followed by the partial immobilization of -SH groups essential for the activating (dehydrogenase) protein portion, at varying inhibitor concentrations. Furthermore, specific dehydrogenase mechanisms have been blocked by iodoacetate, and Gemmill (16) suggested that in yeast respiration the principal site of iodoacetate inhibition appeared to be in the triosephosphate dehydrogenase system. Commoner and Thiman (10) found in the Avena coleoptile respiration an iodoacetate sensitive fraction responsible for a small part

of the total respiration, which apparently was related to growth. After blocking it, growth ceased entirely while oxygen consumption dropped only 10 percent. In bean stem slices, Smith (44) found 70-75 percent inhibition of oxygen consumption and complete inhibition of CO_2 evolution with 1 x 10^{-3} M iodoacetate concentration.

Cell wall plasticity followed by an extension of the wall was considered by Heyn (21) to be the primary factor in elongation, suggesting that the junctions of the cellulose network were probably loosened by weakening of the secondary valences with reversible changes of bound water into free water. X-ray studies showed the presence of amorphous, coalesced microfibrils, these being considered to indicate a large degree of hydration of cellulose units.

The sequence of the cell wall properties observed in passing from the tip through the regions of active cell extension to the mature, non-extending regions, has been expressed in terms of turgor extensibilities as measured from cell shrinkage on plasmolysis. This has been a reasonable assumption since the osmotic pressure of the cells has been found to remain unaltered during elongation. Burström (5), after systematic plasmolysis studies in excised wheat roots, distinguished two phases in cell elongation. The first was one of pure stretching conditioned by increased

elasticity of the cell wall, suggested as being due to increased swelling of intermicellar colloids. At the beginning of the second phase this intermicellar swelling reached its maximum and subsequent increase in wall area was purely by the deposition of new cellulose micellae by active intussusception, largely dependent on the nutrient supply and especially glucose concentration. The results of the observations of Levitt (25) in potato slices held at low temperatures in cyanide solution demonstrated that the estimated additional water uptake probably was due to an increased wall plasticity induced by auxin through the intermediary of the cytoplasm.

Wirth (53), carrying out a carbohydrate fractionation of the cell wall components of Zea mays coleoptile, showed that total cellulose and hemicellulose content ran roughly parallel with total growth in coleoptile length, indicating that during the stretching phase cellulose and hemicellulose were being synthesized and laid down in the cell wall at the same rate as elongation.

The Van't Hoff and Arrhenius temperature coefficient equations formulated from thermodynamic reasoning for simple inorganic systems have been especially valuable in explaining the nature of some factors that limit the rates of biologic processes. Thut and Loomis (48) measured the combined

elongation of a central axis and a young leaf of Zea, the axis of Asparagus, and the leaf blade expansion of Ricinus in field grown plants. The size increments followed the temperature curves up to the limit at which temperature became excessive or moisture supplies limiting. The temperature coefficients of growth in young Ricinus leaves were very clearly two plus; in these, all the growth recorded was probably due to cell enlargement. Such values suggested that the chemical formation and action of auxin or auxins on wall plasticity were the major limiting factors in cell elongation. Chao and Loomis (7) determined the Q_{10} for cell elongation using tissue from Taraxacum scapes, Phaseolus hypocotyl, and Ricinus leaves. In the temperature range from 0 to 25° C., the coefficients were above two. They concluded that the fundamental mechanisms of cell elongation were probably auxin stimulated processes involving complex physico-chemical reactions. Their experiments suggested also that there may be little difference between the mechanisms of division and enlargement, and that minimum amounts of protoplasm are necessary for cell enlargement.

The gradual appearance of vacuoles in parenchymatous cortical cells is a distinct morphological evidence of cell development. Cell vacuolation has been estimated qualita-

tively through histological observations of cross sections made at various root levels. McPherson (32) studied transverse sections of Zea radicles. At 0.5 mm. above the root cap, cortical cells began to vacuolate rapidly, the protoplasm, however, was still fairly dense. At 1.5 mm. a considerable portion of the protoplasm had disappeared or had changed in such a way that it was not stained by haematoxylin. At 5.mm. a large proportion of the cortical cells showed signs of complete maturation. Cormack (11) studied cross sections of Brassica and Lycopersicum radicles. In the former, epidermal cells started to vacuolate at 0.4 mm. above the root cap. At 0.8 mm. further vacuolation of long cells proceeded, and at 1.1 mm. vacuolation of short cells also occurred. In Lycopersicum sections cells began to vacuolate rapidly at 0.6 mm. in all primary tissues.

Vacuolation has been considered to be the result of changes in the protoplasm which render it more permeable, water thus diffusing in at rapid rates. Sifton (42) studied rates of plasmolysis in mesophyll and palisade cells of enlarging leaves of Ledum groenlandicum. In cells which were ready to form vacuoles, plasmolysis occurred rapidly in the mesophyll, but took place only after thirty minutes in palisade cells. The difference in permeability as measured by these variations in plasmolysis rates was considered to be

the factor that brings about vacuolation.

The observation that water also traverses plasma membranes in the absence of pure hydrostatic or osmotic forces or even against such forces, suggesting the possibility of an active participation of the plasma membrane and cytoplasm, led van Overbeek (38) to formulate the hypothesis that active water absorption against a diffusion gradient must be tied up with the supply of energy released in the cytoplasm, and bound up with electrical differences of potential across the plasma membrane.

Levitt (25) showed that on theoretical grounds such energy requirements were well within the metabolic capacity of the tissues concerned, and Kelly (24) using oat coleoptiles established a general parallelism between water uptake and energy produced from respiration (oxygen uptake) under the inhibiting action of a wide variety of inhibitors of oxidative carbohydrate metabolism. She also indicated that such parallelism could be taken as a confirmation of the obligate participation of energy supply and/or substrate produced during carbohydrate metabolism in the active water absorption process; this conclusion was supported by the suppression of water absorption in a nitrogen atmosphere.

Veldstra (50) assumed that any turgescent action whereby the tightness of molecular packing is reduced would increase

permeability. It was held that indoleacetic acid and naphthaleneacetic acid had such a turgescent action on in vitro oleate coacervates. Normal fatty acids with chain lengths from 10 to 18 carbon atoms were tested for activity and it was shown that some activity was exhibited by those acids having the maximum turgescent action on artificial coacervates. He suggested that auxins probably alter either the electric potential or the degree of hydration of the polar groups or both. Guttenberg and Kröpelin (18), using Rhoeo discolor and onion scale epidermis, plasmolyzed cells in 0.5 M urea and 0.5 M urea plus 100 ppm indoleacetic acid. Subsequent deplasmolysis in 0.33 M urea and 0.33 M urea plus 100 ppm indoleacetic acid showed a more rapid rate of recovery with indoleacetic acid, which was taken as evidence of an increased permeability of the cell membrane to water uptake induced by auxin.

Sinnott and Bloch (43) studied cell division in highly vacuolated cells. They found that in the shoot apex such cells were capable of a division rate comparable to that of typical meristematic cells, but in the epidermal-dermatogen tissue of root tips, the type of division characteristic of vacuolated cells was not so commonly found. They explained these observations in terms of the rather sharp transition from the region of actively dividing small cells

at the tip, to the rapidly expanding cells which had ceased dividing.

The appearance of rows of rapidly enlarging cells suggests that the end cells of such rows are inserting themselves among their neighbors. Priestley (39) thought that if such movements occurred, they would be slight and would stop when the plastic matrix of the middle lamella set to a hard mass of insoluble pectates. Adjustments in shape would then take place by a movement of the whole framework of walls. He found that the primary cell walls were very thin, embedded in a fine slime of mucilaginous pectin, and that they were penetrated over wide areas by protoplasm.

MATERIALS AND METHODS

Materials

Young radicle tips of Zea mays L. were chosen for a study of primary growth because of local interest in the plant and its availability, but also because of the relatively large, easily grown roots with their clear-cut zones of division, enlargement and differentiation.

Within the shell of periblem cells of primary maize roots (considered to be immature cortical cells), the division zone, defined as that fraction in which anticlinal divisions are taking place (2), has been further separated into two rather distinct portions. One of these is formed by the group of cells located in the first millimeter above the root cap base, and is characterized by slow growth, small sized and non-vacuolated cells; the other, an adjacent group of cells located in the second millimeter, is characterized by both rapid cell division and enlargement, giving the second millimeter the most rapid rate of total growth. The measurements obtained from radicles grown under the given set of conditions have indicated that division of cortical cells did not occur beyond the second millimeter.

The enlargement zone, defined as that fraction in which only the gain in cell length is accounting for growth, has been separated further into two portions. One of these was formed by the group of cells located in the third millimeter where expansion is relatively rapid. The other portion has been formed by the group of cells located in the fourth millimeter and is distinguished by slowly enlarging cells up the limit at which any gain in cell length became negligible. The growth processes ceased beyond the fifth millimeter and in some experiments both the fifth and the sixth millimeters have been representative of the maturation and differentiation regions.

Sampling, Weighing, and Micro-Chemical Analyses

Uniform corn kernels of the single cross hybrid B.3Gkx [Osf(38-M)] obtained from Dr. G. F. Sprague, were surface disinfected with Arasan and germinated in large covered crystallizing dishes between sterile moist blotters, germination taking place under relatively low microbiological contamination. The dishes were held in a 25 ± 2° C. chamber having a relative humidity of approximately 85 percent. From each lot, the seedlings with the most uniformly developed radicle diamter were selected. The radicles were

frozen in a chamber having a temperature around -20° C. and then sectioned, weighed and dried in minimum time. Sectioning was done with a specially designed cutting instrument 4 x 2.5 x 2.5 cm. having six safety razor blades mounted in a holder, separated by six plates, each 4 x 1.5 cm. and clamped together. The width of each of these plates was 0.9±0.01 mm. and of each blade 0.1±0.02 mm. thus making the separation distance between two adjacent blade edges 1 mm.

In a cold room with temperature around 5° C., using a 30x stereoscopic binoculars and the cutting instrument, each radicle was cut into five 1 mm. segments starting from the base of the root cap. Very slight pressure was required to make the sections. Three replications containing cuts from 250 roots each were made for each of the first five 1 mm. segments. The pieces were sorted into five tared weighing bottles, and the bottles were weighed on a chainomatic balance having 0.00003 g. precision. The weights were calibrated against a U. S. Bureau of Standard set of weights and double weighings of each bottle were made to obtain maximum precision. The samples were dried at 65° C. for 12 hours under vacuum, cooled in a desiccator and reweighed twice. Dry weights were calculated in milligrams and as percentages

of fresh weight.

A separation of colloidal and non colloidal carbohydrate and nitrogen fractions was made by extracting the dry sample with 80 percent ethyl alcohol following the procedure described by Loomis and Shull (28). Each dry sample was transferred to a 10 x 50 mm. Whatman extraction thimble and continuously refluxed in 30 ml. of 80 percent ethyl alcohol during 24 hours in a micro soxhlet extraction apparatus. After completion of extraction, the sample was separated into extract and residue, the former containing the water and fat soluble non-colloidal substances while the residue contained the remaining insoluble and colloidal substances, the dry weight of which was estimated after drying it in a 100° C. oven for 24 hours. The excess alcohol in an aliquot of the extract was evaporated off under reduced pressure, the flask was cooled, then the solution was made acid to methyl red with 10 percent acetic acid and 2 to 4 drops of 1 percent invertase solution were added with a little toluene. The volumetric flasks were allowed to stand overnight, and before proceeding with total reducing sugars determination, the temperature of the solution was adjusted to 20° C.

Total reducing sugars were determined on a 5 ml. aliquot of each extract by the ceric sulfate method (19).

A 5 ml. sample was pipetted into an 8 inch test tube and 5 ml. of alkaline ferricyanide solution added. The test tubes were mounted in a rack and boiled in a water bath for fifteen minutes ± 1 second, then cooled to room temperature and to each one, 5 ml. of 5 N sulfuric acid was added. The titration was completed with 0.01 N ceric sulfate solution until the green color changed to a golden brown under 5-7 drops of setopaline-C indicator solution. The ceric sulfate solution was standardized with a known quantity of glucose.

The soluble nitrogen determination was made with the micro-Kjeldahl procedure described by Johns (22). A 15 ml. aliquot was transferred to a 100 ml. micro-Kjeldahl flask and evaporated under reduced pressure to 1-2 ml. Each sample was digested with 25 drops conc. sulfuric acid. To hasten the digestion 0.1-0.2 g. of a 1:1 mixture of potassium sulfate and copper sulfate was added. During the digestion one or two drops of 30 percent hydrogen peroxide were added dropwise to hasten the combustion of the carbonaceous material. When the digestion was completed after 12 to 15 minutes the flask was allowed to cool and its content was diluted with 5 ml. of CO_2-free distilled water.

The distillation apparatus was similar to the original one of Pregl. During the digestion of the samples the

distillation apparatus was thoroughly steamed out with the steam generator heated with a low flame and the cooling water not connected to the condenser. When the first distillation was to be started, the plug in the top of the steam generator was removed, the cooling water started through the condenser and a test tube 20 x 150 ml. containing 5 ml. of 2 percent boric acid and two drops of methyl indicator mixture was supported on a plate at such a height that the condenser tube reached nearly to the bottom. The flask containing the digested sample was poured into the funnel, the flask rinsed twice with 3 ml. of CO_2-free distilled water, and the washings poured into the funnel. An excess of a saturated solution of sodium hydroxide was dropped into the funnel and allowed to run slowly to the bottom of the distillation chamber. Steam was passed through the sample until the volume in the collection tube doubled, then the tube was removed and the delivery tube was washed off with a small amount of distilled water. The titration with 0.02 N HCL was completed in the test tube. For each set a blank analysis was run which was equivalent to no more than 0.02 ml. of the 0.02 N acid.

Respiration Measurements

The respiration rates and respiratory quotients characteristic of the primary corn root meristematic regions

were studied by placing a representative sample of each growth zone in the vessel of a constant volume Warburg respirometer flask held at 30.00±0.25° C. and by measuring O_2 uptake and the CO_2 evolution with the Warburg direct method (49). Germinated corn seedlings with radicle diameter well within the allowable limits for adequate gas diffusion were quickly immersed in a freshly prepared and filtered one percent calcium hypochlorite solution and washed thoroughly with sterile tap water. Low microbiological contamination was maintained and experiments of several hours duration were permitted. The radicles were randomized and cut into three 2-mm. segments starting from the root cap base, thus including in each cut the regions of cell division and enlargement, cell enlargement, and cell differentiation respectively. The cuts were transferred directly to the solution in the vessel of the respiration flask and placed in the constant temperature bath. The induction period given to the excised roots to adjust their metabolism to the new conditions varied with the type of experiment. Between readings, the system was shaken at one hundred 2 cm. strokes per minute, to be certain that the results were independent of the rate of shaking. At the end of each determination, dry weight and total nitrogen were estimated. The final results were

expressed as microliters (mul.) O_2 taken up per mg. dry weight per hour and as mul. O_2 taken up per mg. tissue nitrogen per hour. The same expressions for CO_2 evolution were used. Respiratory quotients were calculated as the ratio between CO_2 produced and O_2 consumed. The observations were analyzed statistically and the mean, standard deviation and standard error of the mean at the 5 percent probability level were calculated.

Tissue Cultures

Meristematic root tip segments representing the three growth zones were grown in solution culture under sterile conditions following techniques similar to those of Robbins (40). The germinated corn seedlings were disinfected as for the respiration studies, and sorted in groups of 10 in sterile petri dishes containing 3 ml. sterile double distilled water with a drop or two of a 1 percent calcium hypochlorite solution. The three growth zones were cut from each radicle with the cutting tool, which was sterilized by dipping it in 95 percent ethanol and burning off the ethanol. The three segments were transferred individually to separate culture media with a loop needle of chromium wire. The needle was

sterilized in a flame and allowed to cool before touching the root fragment. All work involved in transferring root segments was carried out in a transfer chamber and contaminations were not frequent. Whenever a flask became contaminated it was discarded. The excised zones were grown individually in 3 ml. of nutrient solution in 30 ml. erlenmeyer flasks of pyrex glass held at 30.0±2.0° C. The flasks were mounted in a wrist-hand action shaker operating at 100 shakes per minute, thus permitting good aeration.

The basic medium for these experiments was a combination of White's (52) and Bonner's(4) nutrient solutions with the following formulation.

	mg	Chemical lot no.
$Ca(NO_3)_2$	100.0	Bakers - 41
KNO_3	80.0	Bakers - 26
$MgSO_4.7H_2O$	35.0	Merck - 18327
KCL	65.0	Merck - 42994
KH_2PO_4	12.5	Bakers - 63
H_3BO_3	1.5	Bakers - D327
$ZnSO_4.7H_2O$	1.5	Bakers - D279
$MnSO_4.4H_2O$	4.5	Bakers - 27
Thyamine hydrochloride	0.1	General Biochem, Inc - 18079
Glycine	2.0	Pfanstiehl - 583
Pyridoxine	0.1	

	mg	Chemical lot no.
Nicotinic acid	0.5	Merck - 52871
Fe tartrate	1.5	Sargent

Redistilled water up to 1000 ml

2 percent by weight of c.p. sucrose

The nutrient media were sterilized in the autoclave at 15 pounds pressure for 15 minutes.

At the end of each experiment, the gain in length of the segments and the dry weights of the segments were taken as indicators of growth, unless otherwise stated.

REGIONS OF GROWTH

The specific contribution given by the increase in the number and/or length of cells to the longitudinal expansion occurring simultaneously in all sections of the growing region of germinating Zea mays radicles was estimated for each of the five zones of growth under study. Information concerning cell length position along 1 mm. sections was derived from the cell length determinations made by following one row of cells in the cortex, usually the third or fourth row from the epidermis. These data are shown in Table 1.

The first millimeter section in radicles grown at 25° had elongated 0.2 mm. into the region originally occupied by the second section. In the latter, the first 250 mu contained 21 cells of 12 mu. each; therefore, 0.2 mm. contained 0.2 x 21/0.250 = 17 new cells. The first section contained at the beginning 90 cells, and adding the newly formed cells there were estimated at the end of three hours to be a total of 107 cells. The specific rate of cell division was calculated to be 0.055 cells per cell per hour and the time necessary for one complete cell division was found to be around 18 hours.

Table 1. Estimates of total growth, length of cortical cells, specific rates of cell division, and rates of enlargement in consecutive 1 mm. segments of the maize root tip at 25° C.

Mm. from tip	Mm. growth in 3 hours	Cell length	No. of cells per mm.	Specific rate of cell div.	Time for one cell gen.	Rate of enlargement, percentage
1	0.2±.03	11±.02	91	0.055	18 hr.	0.0
2	0.9±.08	19±.10	52	0.126	8 hr.	71
3	0.6±.12	53±1.5	19	0	— -	60
4	0.3±.03	70±1.0	14	0	— -	30
5	0.0	96±1.5	10	0	— -	0.0

Since the average length of the cells did not change significantly during this time, this growth is arbitrarily considered to be due to cell division alone. Cell number in this section was estimated to have increased 19 percent, while section length increased 20 percent.

In the second millimeter of the root, cell number increased 33 percent from 52 to 69, but section length increased 90 percent and average cell length increased 72 percent from 11 to 19 mu. Not only was cell division more rapid in the second millimeter than in the first, but the cells also increased rapidly in length. This increase began slowly, with cells in the first quarter of the section averaging only 12 mu, and then increasing to 19, 24 and 32 mu in succeeding quarters. Cell division, as determined by mitotic figures, was concentrated in the first half of the second section and was not observed in the fourth quarter or in any succeeding section.

The second millimeter section had elongated 0.9 mm. by the end of three hours into the region originally occupied by the third millimeter. The third millimeter contained an average of 19 cells, so that the number attributable to the second section at the end of the period was 0.9 x 19 = 17 new cells, plus 52 original = 69 total. A specific cell division rate of 0.092 cells per

hour is indicated, with the time for one cell generation about 10 hours. This calculation, however, includes many cells which had been pushed past the third quarter of the second section where cell division stopped. An estimation of actual cell division rates, which is probably still low, can be made by arbitrarily assigning all cell division to the first half of the section. Our numbers are now 37 plus 17 cells, and the specific cell division rate 0.126 with one cell generation in about eight hours.

While these generation times are not considered to be minimum, it is obvious that cell division in a root meristem is much slower than, for example, in a bacterial culture where generation times of one-half to one hour are estimated. The difference is attributed to translocation rates and to competition among dividing cells for available foods. It seems significant that the rate of cell division near the back end of the division zone, but nearest the endosperm food supply, was more than twice that of the so-called typical meristem of the first millimeter.

Evidence on division in vacuolated cells was not obtained directly because of the short sections ($\pm$0.1 mm.) involved. It may be pointed out, however, that the slowdown in division rates was not observed until the average length shown by the apical cells was approximately doubled.

To the original 52 cells occupying the second millimeter were added 17 new cells by division, making a total of 69 cells spread over 1.9 mm. Because we have considered as cell division any enlargement required to bring the daughter cells to the average length (11 mu) of the cells in the apical section, 17 x 11 = 187 mu of this total growth is assigned to cell division and 900 - 187 = 713 mu to cell enlargement as a separate growth factor. These figures show an average enlargement of 10.3 mu per cell for the three hour period or 71 percent.

In the third millimeter of the root tip cell enlargement alone accounted for a 60 percent gain in section length in three hours at 25° C. By the end of this period the third millimeter became the fourth millimeter because of growth in the first and especially in the second section. The 19 cells originally occupying 1 mm. or 1000 mu, by the end of three hours were spread over 1600 mu; and the increase in the average length of cells was (1600/19)-53 = 31 mu in three hours.

Since initial growth in the fourth millimeter was about one-third that of the third section, we can assume that this figure is low for the instantaneous rate of enlargement in the third millimeter. The fourth millimeter showed a total enlargement of 30 percent. The 14 cells

originally occupying 1 mm. or 1000 mu, by the end of three hours were spread over 1300 mu; therefore, the increase in the average length of cells was (1300/14)-70 = 23 mu in three hours.

Within the three hour period this section was displaced to the fifth and sixth millimeter position in which no growth occurred. Obviously the enlargement recorded was fairly rapid, but limited to the early part of the three hour period.

CHEMICAL ANALYSES OF DEVELOPMENTAL ZONES

In the chemical determinations made for each of the first five 1 mm. growth zones of Zea mays radicles, both the central cylinder tissues and the shell formed by the endodermis, cortical layers and epidermal tissues were included since separate estimations were impracticable. The chemical components estimated for each 1 mm. segment included, therefore, the fractions of both central cylinder and shell at the moment of sectioning, but since approximately 89 percent of the volume of the root segments in the meristematic region is parenchymatous cortex, the specific constituents characterizing each growth zone were essentially those of a single simple tissue.

In maize radicles grown at 25° C. the green weight per millimeter nearly doubled between the first and second sections (Table 2) while the rounded tip was being eliminated and average root diameter increasing. Further but slower increases in diameter brought the weight of the fifth section to 141 percent of that of the second. Green weight per unit cell length, in contrast, increased nearly 22 times between the first and fifth millimeters, and the

size of individual cells increased logarithmetically with their section position (Table 3).

A comparison of the dry matter in segments within the same radicle showed that the second millimeter, the region of most rapid growth, yielded the largest quantity of dry matter (Table 2). This result again was confounded with section diameter. The round tipped terminal section was only about 60 percent as large as the second section, which in turn was considerably smaller than the fifth. The high percentage of dry matter in the tip segment, as contrasted to total quantities, indicates that the increased hydration shown back of the second section had already begun in this section. Dry matter per unit cell length increased 7 times in the 5 mm. (Table 3) against 22 times for fresh weight. The difference represents accumulation of water in vacuoles, etc.

Available Compounds

Sugars move to the growing tip from the endosperm and are used in respiration and metabolism as they move. It is not surprising, therefore, to find the highest sugar contents and percentages in the proximal sections. If the determination of total reducing substances was not too much

Table 2. Chemical composition of consecutive 1 mm. segments of maize root tips grown at 25° C.

Mm. from tip	Units	Fresh weight	Dry matter	Alcohol insoluble matter	Total sugars	Total nitrogen	Alcohol soluble nitrogen	Insoluble nitrogen as protein (x6.25)	A
1	Gamma	655	128	112	5.5	12.50	1.7	67.5	5
	% Green wt.		19.6	17.1	0.84	1.91	0.26		0
	% Dry wt.				4.30	9.80	1.30	53.3	4
2	Gamma	1110	180	140	10.0	16.50	4.2	76.8	7
	% Green wt.		16.2	12.6	0.90	1.48	0.38		0
	% Dry wt.				5.55	9.20	2.34	43.0	3
3	Gamma	1190	154	92	12.5	13.00	4.8	51.3	5
	% Green wt.		12.9	7.7	1.05	1.09	0.40		0
	% Dry wt.				8.15	8.45	3.12	33.4	3
4	Gamma	1350	105	69	14.5	7.40	2.4	31.2	2
	% Green wt.		7.8	5.1	1.07	0.55	0.18		0
	% Dry wt.					7.05	2.48	27.3	2
5	Gamma	1570	100	50	18.5	4.50	1.50	18.7	.
	% Green wt.		6.4	3.2	1.18	0.29	0.095		0
	% Dry wt.				18.50	4.50	1.50	18.7	.

*Residue from acid hydroloysis minus ash.

tion of consecutive 1 mm. segments of maize root tips

ter	Alcohol insoluble matter	Total sugars	Total nitrogen	Alcohol soluble nitrogen	Insoluble nitrogen as protein (x6.25)	Ash	Acid hydroly. materials	Cellulosic materials*
	112	5.5	12.50	1.7	67.5	5.5	17.0	5.5
.6	17.1	0.84	1.91	0.26		0.84	2.60	0.84
		4.30	9.80	1.30	53.3	4.30	13.25	4.30
	140	10.0	16.50	4.2	76.8	7.0	23.0	14.0
.2	12.6	0.90	1.48	0.38		0.63	2.07	1.26
		5.55	9.20	2.34	43.0	3.90	12.80	7.80
	92	12.5	13.00	4.8	51.3	6.0	15.5	14.0
.9	7.7	1.05	1.09	0.40		0.51	1.30	1.18
		8.15	8.45	3.12	33.4	3.90	10.10	9.10
	69	14.5	7.40	2.4	31.2	2.5	11.0	13.0
.8	5.1	1.07	0.55	0.18		0.19	0.82	0.97
			7.05	2.48	27.3	1.38	10.50	12.4
	50	18.5	4.50	1.50	18.7	.5	7.1	15.0
.4	3.2	1.18	0.29	0.095		.09	0.45	0.96
		18.50	4.50	1.50	18.7	.50	7.10	15.0

minus ash.

Table 3. Chemical composition in micrograms per unit cell length in the first five 1 mm. sections of maize root tips grown at 25° C.

Mm. from tip	Fresh weight	Dry matter	Alcohol insol. matter	Total sugars	Total N	Alcohol soluble N	Inso N (x6.
1	7.20	1.41	1.23	.06	0.14	0.02	0.75
2	21.40	3.46	2.70	.19	0.32	0.08	1.48
3	62.80	8.10	4.84	.65	0.68	0.25	2.70
4	96.50	7.50	4.92	1.04	0.52	0.17	2.22
5	157.00	10.00	5.00	1.85	0.45	0.15	1.87

in micrograms per unit cell length in the first
f maize root tips grown at 25° C.

cohol sol. tter	Total sugars	Total N	Alcohol soluble N	Inso l. N (x6. 25)	Ash	Acid hydrol. materials	Cellulosic materials
.23	.06	0.14	0.02	0.75	.06	0.18	0.06
.70	.19	0.32	0.08	1.48	0.13	0.44	0.27
.84	.65	0.68	0.25	2.70	0.32	0.81	0.74
.92	1.04	0.52	0.17	2.22	0.18	0.78	0.93
.00	1.85	0.45	0.15	1.87	0.15	0.71	1.50

confounded by amino and organic acids, the determinations (cf. sugar as percent green wt., Table 2) suggest that sugars should not have been limiting for cell development, even at the root tip.

Nitrogenous materials also were being moved from the endosperm, but there is no gradient in Table 2 from which we can select a mobile fraction. Soluble (non-colloidal) nitrogen was 4 times higher in the third section than in the fifth, then tapered slightly toward the tip. These figures might be considered to indicate functioning protophloem to the third millimeter in which nitrogenous materials were moving at a concentration not detectable by mass tissue analysis. The relatively high concentrations of soluble nitrogen on a per cell unit (Table 3) as well as a green weight basis came in the third section where growth was limited to cell enlargement, but where proteins were still being synthesized.

The Van Slyke amino nitrogen determinations were probably too low, since free alpha amino groups may be blocked by other alcohol soluble compounds (51), reducing the amino nitrogen recoveries. Loomis (26) has shown that materials in the soluble nitrogen fraction other than alpha amino compounds may be physiologically active in protein

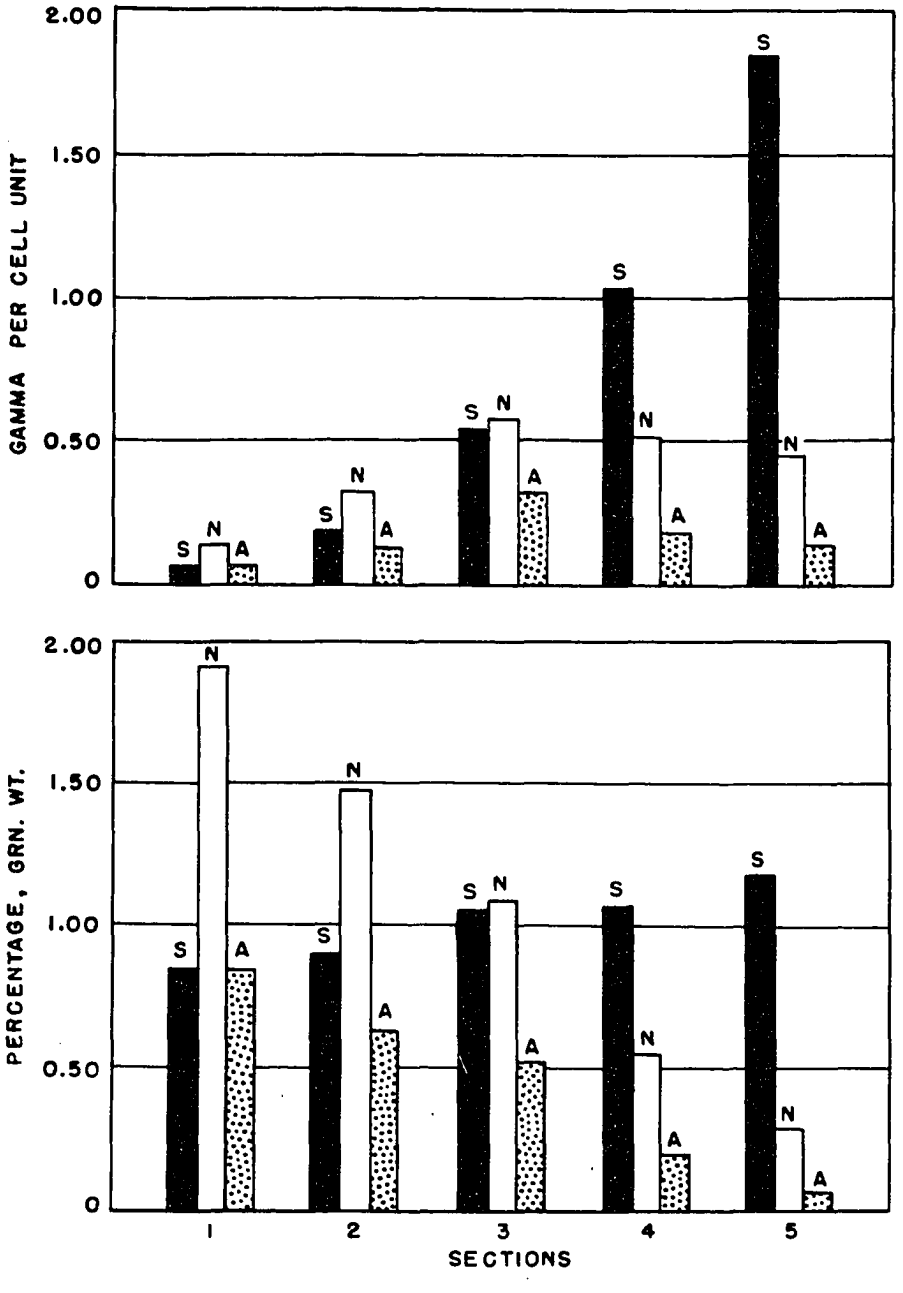


Figure 1. Total reducing substances (S), nitrogen (N), and ash (A) in percentage of the green weight and in gamma per cell unit of consecutive 1 mm. segments of maize root tips grown at 25° C.

synthesis and since the amino nitrogen determinations made in each segment appeared to be consistently correlated with soluble nitrogen, the specific growth characteristics in primary corn root meristems may be related to the distribution of total soluble nitrogen.

Proteins and Ash

The concentration of the protein fraction characterizing the apical five 1 mm. sections of primary corn roots was estimated by converting the difference between total and soluble nitrogen into crude protein. The conversion factor was 6.25. In the alcohol insoluble protein nitrogen, colloidal proteins, which could be considered to have already been used in growth, are included. The extent of this fraction could be indicative of the type of growth that took place in each of the five 1 mm. section of primary corn roots, provided other factors remained unchanged. In the first millimeter, the colloidal, protein fraction was high and amounted to 53.0 percent of the dry weight; in the second millimeter the concentration had dropped to 43.0 percent of the dry weight; in the third millimeter to 33.4 percent, and in the fourth to 27.3 percent. Finally, the region of differentiation

was characterized by a concentration of colloidal, protein nitrogen of 18.7 percent of the dry weight.

The relatively large concentration of colloidal protein found in the first and second millimeter sections may be related to minimum tendencies for swelling and vacuolation, thus maintaining the physico-chemical conditions most favorable for division. Enzymatic systems operating in these zones could immobilize rapidly, mostly through the formation of new protoplasm, a large part of the soluble nitrogen available. These suggestions would be in accord with the finding that in primary corn roots the processes resulting in the formation of new cells were confined to the meristematic tissues occupying the first and second apical millimeter regions.

Although colloidal nitrogen per section decreased uniformly from the first to the fifth millimeter, the content per unit of cell length increased nearly four times between the first and third sections. If we compare the corresponding cell lengths (Table 1) we see that protein concentrations were maintained across the second millimeter, in most of which cells were still dividing, but dropped slightly in the third millimeter of cell enlargement only. It seems highly significant, however, that the protein

content of these enlarging cells should have nearly doubled over that of the second section (Table 3). Between the third and fifth sections cell length nearly doubled while protein and total nitrogen per cell unit decreased. If hydration alone had been operative the nitrogen content on the unit basis would have remained unchanged. The data indicate that some of the nitrogen for cell division and enlargement was obtained by digestion of proteins as close to the tip as the fourth and fifth millimeters. They show also the possibility of cell enlargement with either increasing protein (3rd section) or decreasing protein (4th section). The last, which was shown clearly in the later stages of dandelion stipe elongation (7) may not have occurred in this primary meristem, since elongation lasted for only a short time in the fourth millimeter and the protein digestion may have taken place during the latter part of the three hour observation period used.

The mineral content of the growth zones was determined gravimetrically, carrying out microincineration at 600° C. The ash weighings were made on a Kuhlman microbalance having a 50 gamma sensitivity factor per scale division and two weighings by the single swing method were made with an

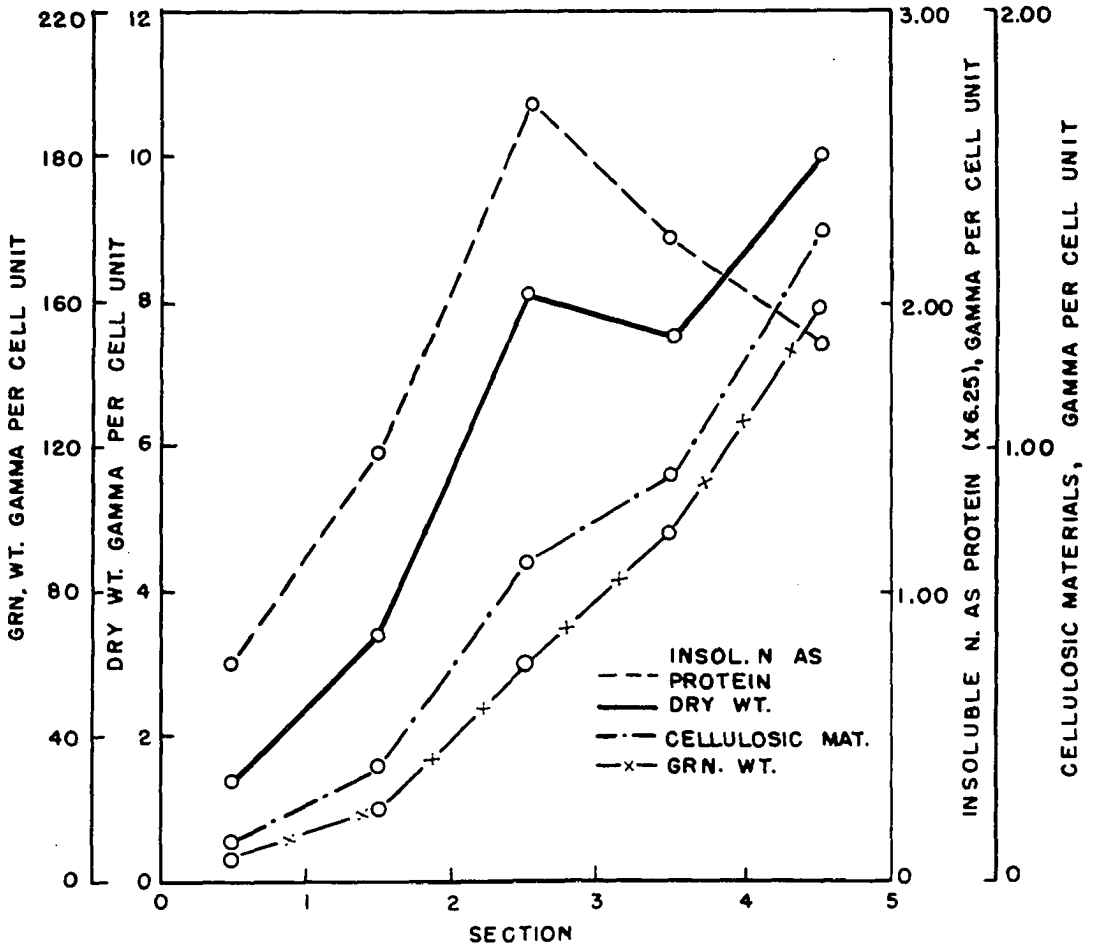


Figure 2. Distribution of differentiation products, in gamma per cell unit, of consecutive 1 mm. segments of maize root tips grown at 25° C.

interval of two to three minutes between weighings, to obtain approximately 2 gamma precision. The ash content was calculated in gamma per millimeter section and also as a percentage of the fresh and dry weights. Ash accumulated in the apical 1 mm. section was found to be nine times larger than the ash content of the fifth millimeter. Qualitative colorimetric determinations indicated that ash in the first and second millimeter was rich in potassium, especially in the second millimeter, while in the third and fourth millimeter calcium predominated over either potassium or magnesium. The qualitative test of the ash of the fifth millimeter did not yield reasonable indications, probably due to the small ash content of this zone, although there was some evidence of calcium accumulation.

Cell Wall Constituents

Under the subheading of "hemicelluloses" were included all the alcohol insoluble substances from each 1 mm. sample which were hydrolyzed by 1 + 20 hydrochloric acid in an open erlenmeyer flask autoclaved at 15 pounds pressure for one hour. Total reducing substances were determined by the ceric sulfate method and calculated as glucose anhydrid. The conversion factor to hemicelluloses

was 0.88 and the results were expressed as percentage of the dry weight. The slope of the hemicellulose distribution graph did not vary significantly along the first and second mm. section. At the border line of the zone of division and enlargement, and rapid enlargement, the relative rate of change was equivalent to a decrease of 65 percent and between the regions of rapid and slow enlargement the slope rate of change was found to be null. At the borderline of the region of slow enlargement and differentiation the slope dropped around 80 percent.

The results of the direct cellulose determinations were invalidated by unknown amounts of hydrolyzed proteins and other reducing substances which were confounded with celluloses. On the other hand, since lignin and other differentiation products are present probably in non-detectable concentrations in these slightly differentiated meristematic regions, the concentration of cellulosic materials in each one of the first five 1 mm. sections couldbe represented by the weight difference between the residue from the acid hydrolysis and total ash.

Cellulosic materials increased from the terminal section to the region of cell differentiation, fifth and sixth mm. The relative deposition of cellulosic materials

varied from one section to another. In gamma per section there was no increase between the second and fifth sections, but as a percentage of total dry weight, and especially on a per cell basis, cellulosic materials increased rapidly in the older, more differentiated cells.

GROWTH IN VITRO

An examination of the chemical structure of intact tissues and the description of its relation to changes in meristematic activity may reveal the nature of the sequence of the cell division and enlargement processes. A study of the effects of induced chemical changes as sections develop under controlled conditions may also furnish information on that point. The clearly defined growing regions at different radicle levels of primary maize roots offered an opportunity for *in vitro* treatments of distinct meristematic regions. In view of recent interest on auxin action, a number of auxin concentrations were tested in separate 2 mm. sections suspended in aerated nutrient solution (4, 52). The data on growth and chemical composition after a 24 hour growth period are shown in Tables 4 and 5.

Indoleacetic acid was related to the developmental changes in isolated meristematic sections in at least three different ways. In the division zone, a 1×10^{-6} M/l induced a retarding action in the gain of both fresh and dry weights and in section length, while the metabolic

activities of the enlargement zone appeared to be completely inhibited and the sections killed with fresh and dry weight losses. In sections suspended in 1 x 10^{-7} and 1 x 10^{-8}M/l abnormal meristematic activities such as branching, callous formations and bending were observed. Finally, sections treated with 1 x 10^{-9}M/l showed a relatively large growth stimulation as manifested by gain in section length and fresh weight. These zones were largely vacuolated and had small root diameter.

Burström (5) found in isolated wheat root segments, retardation and stimulation under the same auxin concentration at different intervals after application and at different stages of meristematic development. Furthermore, the findings of Chao and Loomis (7) that protein and hormone synthesis within the cell stimulated enlargement was indicative that auxins alone were unable to carry out relatively large growth stimulation without accompanying protoplasm synthesis. Since in the apical 2 mm. section of maize root tips most of the protein condensation reactions were found to occur, (cf. Table 2), a relatively large growth response due to auxin could be anticipated. In the apical 2 mm. section, the growth response was found to be larger than that of the third and fourth mm., thus yielding support to the previous inference.

Total sugars accumulated in the sections treated with

Table 4. The *in vitro* effect of indoleacetic acid on the growth and chemical composition of the apical 2 mm., meristematic portion, of primary maize roots grown in nutrient solution at 30° C.

I.A.A. M/l	Length in mm.	Diameter mm.	Gamma fresh wt.	Gamma dry wt.	% dry wt.	Total sugar, % gm. wt.	Soluble nitrogen, % gm. wt.	Alcohol insoluble matter,
At beginning of Experiment:								
Check	2.0	0.8	1800	310	17.2	.78	0.25	257
At end of 24 hours:								
Check	7.7	1.0	4300	610	14.2	5.80	0.96	260
$1x10^{-6}$	4.5	0.8	2800	374	13.5	5.60	1.12	—
$1x10^{-7}$	9.5	1.2	3400	420	12.3	3.18	0.52	—
$1x10^{-8}$	11.3	0.6	6350	620	9.8	2.57	0.49	394
$1x10^{-9}$	15.4	0.7	8275	760	9.2	1.54	0.33	540

Table 5. The *in vitro* effect of indoleacetic acid on the growth and chemical composition of the enlargement zone (mm. 3 and 4) of primary maize roots grown in nutrient solution at 30° C.

I.A.A. M/l	Length in mm.	Diameter mm.	Gamma fresh wt.	Gamma dry wt.	% dry wt.	Total sugar, % gn.wt.	Soluble nitrogen % gn.wt.	Alcohol insoluble matter,
At beginning of Experiment:								
Check	2.0	1.2	2540	260	10.2	1.10	0.35	165
At end of 24 hours:								
Check	2.0	1.4	2640	300	11.3	3.80	2.40	171
$1x10^{-6}$*	2.8	0.8	1870	130	6.9	0.17	0.15	—
$1x10^{-7}$	3.8	1.6	3120	360	11.5	2.20	0.60	—
$1x10^{-8}$	6.3	1.5	5900	480	8.2	1.60	0.47	337
$1x10^{-9}$	7.6	1.3	3940	300	7.6	1.40	0.20	230

*Section killed by excess hormone.

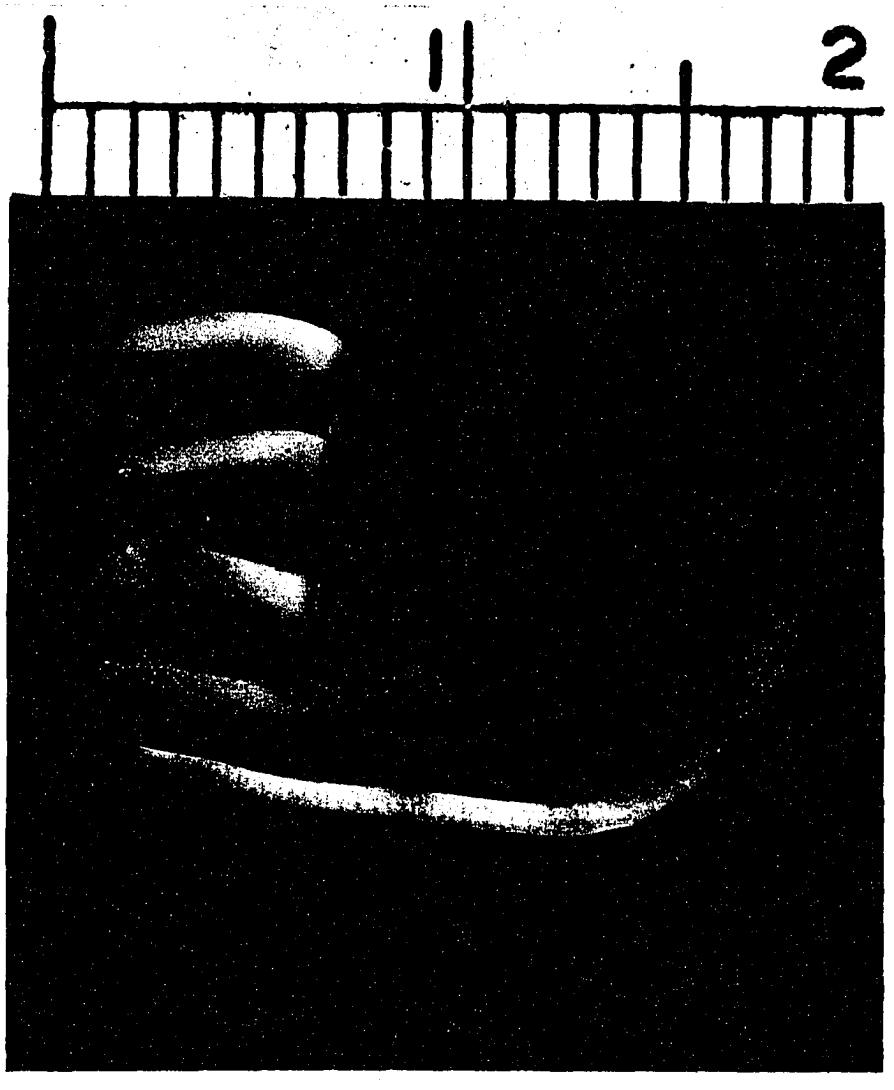


Figure 3. The growth *in vitro* of the division zone (mm. 1 and 2) of primary maize roots grown in nutrient solution with varying concentrations of indoleacetic acid. From top to bottom, length in mm. at the end of 24 hours of the check, 1×10^{-6}, 1×10^{-7}, 1×10^{-8} and 1×10^{-9} M/l of indoleacetic acid.

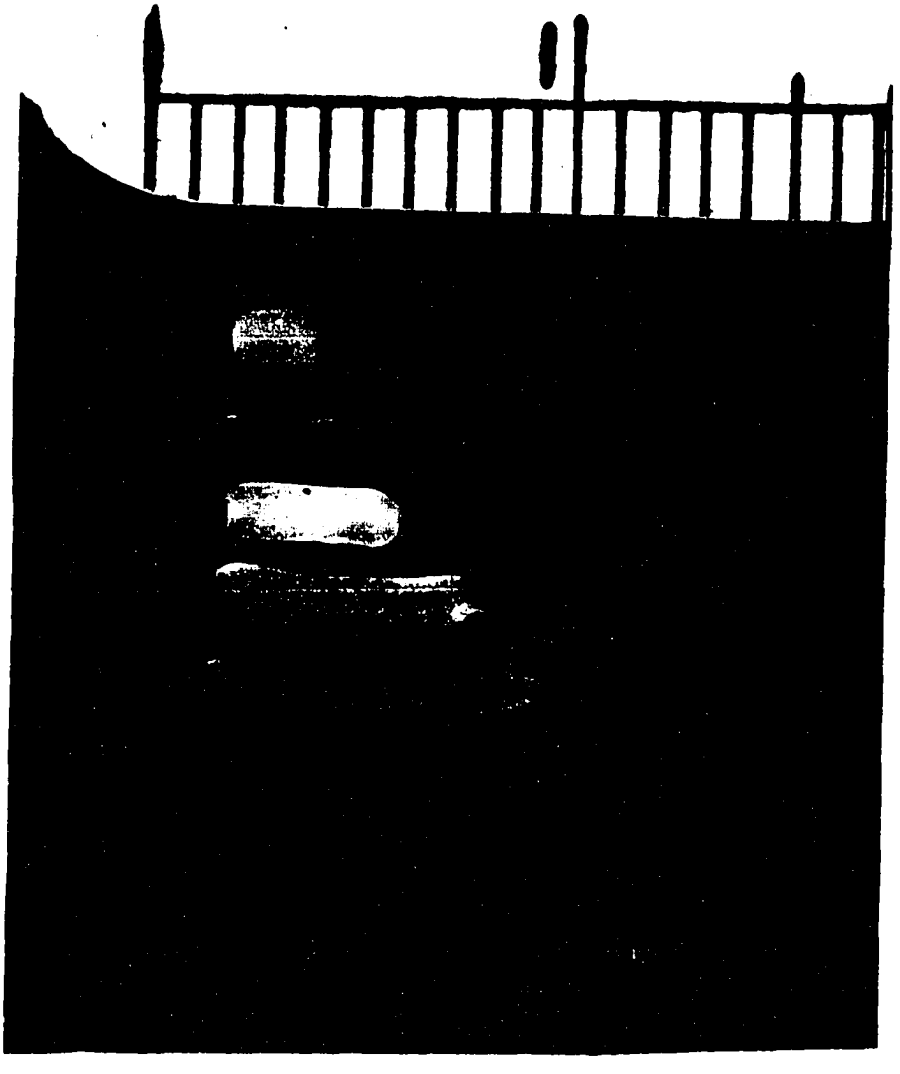


Figure 4. The growth *in vitro* of the enlargement zone (mm. 3 and 4) of primary maize roots grown in nutrient solution with varying concentrations of indoleacetic acid. From top to bottom, length in mm. at the end of 24 hours, of the check, 1×10^{-6}, 1×10^{-7}, 1×10^{-8} and 1×10^{-9} M/l indoleacetic acid.

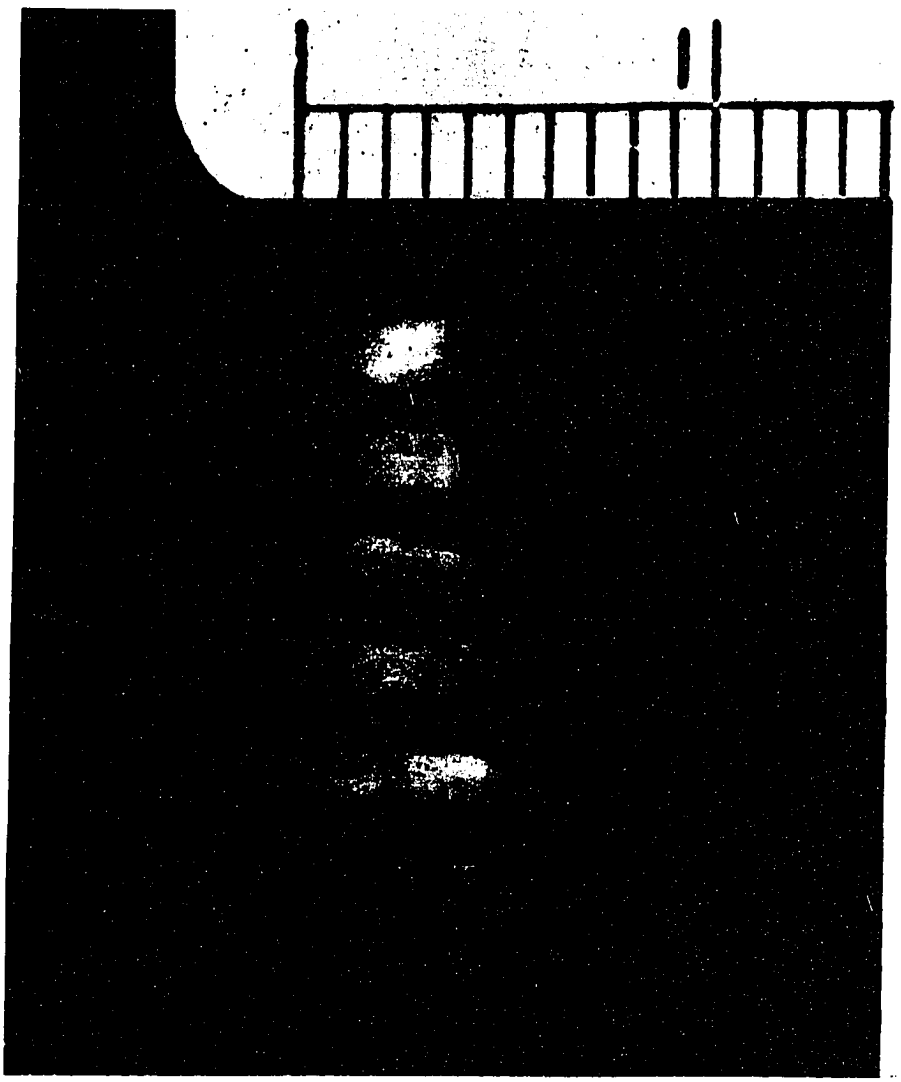


Figure 5. The growth *in vitro* of the differentiation zone (mm. 5 and 6) of primary maize roots grown in nutrient solution with varying concentrations of indoleacetic acid. From top to bottom, length in mm. at the end of 24 hours of the check, 1×10^{-6}, 1×10^{-7}, 1×10^{-8} and 1×10^{-9} M/l indoleacetic acid.

1×10^{-7} and 1×10^{-8}M/l. The percentages of the fresh weight were found to be two and three times larger than those found in intact root sections. The division and enlargement zone treated with 1×10^{-9}M/l showed sugar concentration approaching standard level. In the division zone the relative deposition of alcohol insoluble material increased two times, and in the enlargement zone it increased one third.

The sucrose in the nutrient solution was replaced by glucose or glucose-1-PO_4. 2 mm. meristematic sections were suspended in 3 ml. aerated nutrient solution plus 1×10^{-9}M/l indoleacetic acid. The pH was adjusted with citrate buffer between the range 4.0 to 6.5 at 0.5 intervals. The flasks were incubated at 30° C. Growth variations at the end of 18 hours are shown in Table 6.

The relatively largest gain in section length occurred in the cultures containing sucrose, followed very consistently by the gain in section length which took place in cultures containing glucose as the carbon source. No growth took placein the cultures containing glucose-1-PO_4 at pH other than 5.0. The apparent inability of the meristematic maize root systems to utilize glucose-1-PO_4 at pH other than 5.0 may be considered in connection with the blocking of phosphatase systems. It has been shown (47) that these enzymes operate

under a rather restricted hydrogen ion level. Around pH 5.0 the mono-hexose phosphatase system operating in maize roots probably reached a minimum activity level, and glucose-1-PO_4 diffused in on a concentration gradient. Once inside the protoplasm, the compound probably was incorporated in the usual carbohydrate pathway. At pH 5.0, sections grown in glucose-1-PO_4 showed as good a growth response as those grown in sucrose and better than those grown in glucose.

Table 6. The *in vitro* influence of different sugars on the development of the zone of division (mm. 1 and 2) and enlargement (mm. 3 and 4) of primary maize roots grown at 30° C. in nutrient solution adjusted at different pH. Length of 2.0 mm. segments at the end of 18 hours.

Substrate	Region	pH					
		4.0	4.5	5.0	5.5	6.0	6.5
2% glucose	Division	2.7	3.0	4.5	6.5	6.8	7.0
	Enlargement	2.5	3.0	4.0	5.8	6.0	6.3
2% glucose-1-PO_4	Division	2.0	2.0	7.0	2.2	2.0	2.0
	Enlargement	2.0	2.2	5.0	2.2	2.0	2.0
2% sucrose	Division	3.5	4.0	7.0	7.9	8.3	8.5
	Enlargement	3.0	4.0	6.0	7.0	7.0	7.2

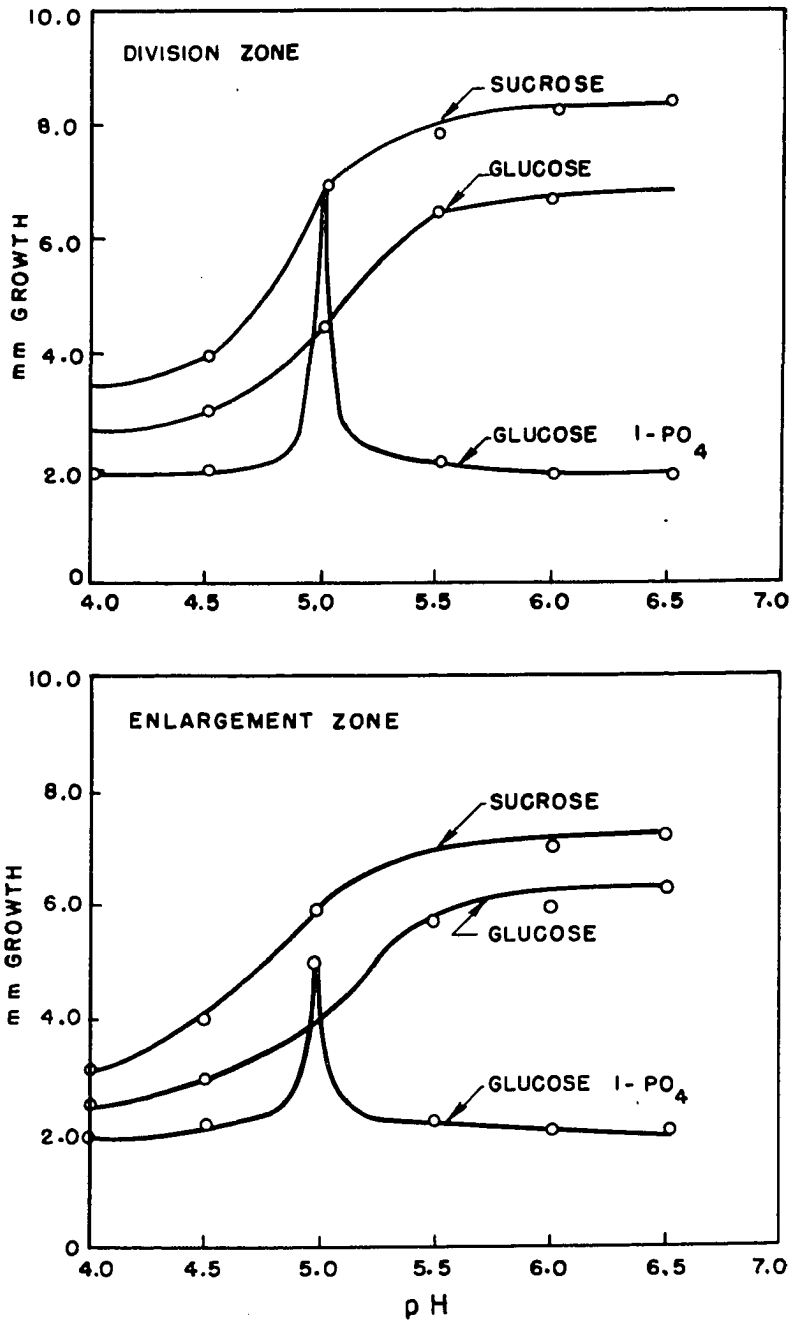


Figure 6. The length after 18 hours of the division (mm. 1 and 2) and enlargement (mm. 3 and 4) zones of primary maize roots grown in vitro in nutrient solution with different carbon sources, in the pH range from 4.0 to 6.5.

Figure 7. Growth *in vitro* of the division (right column) and enlargement (left column) regions of maize roots at three pH levels with three carbon sources. From top to bottom (by groups of three), pH 4.0, 4.5 and 5.0; within groups, top to bottom, 2% glucose, 2% glucose-1-PO_4, 2% sucrose.

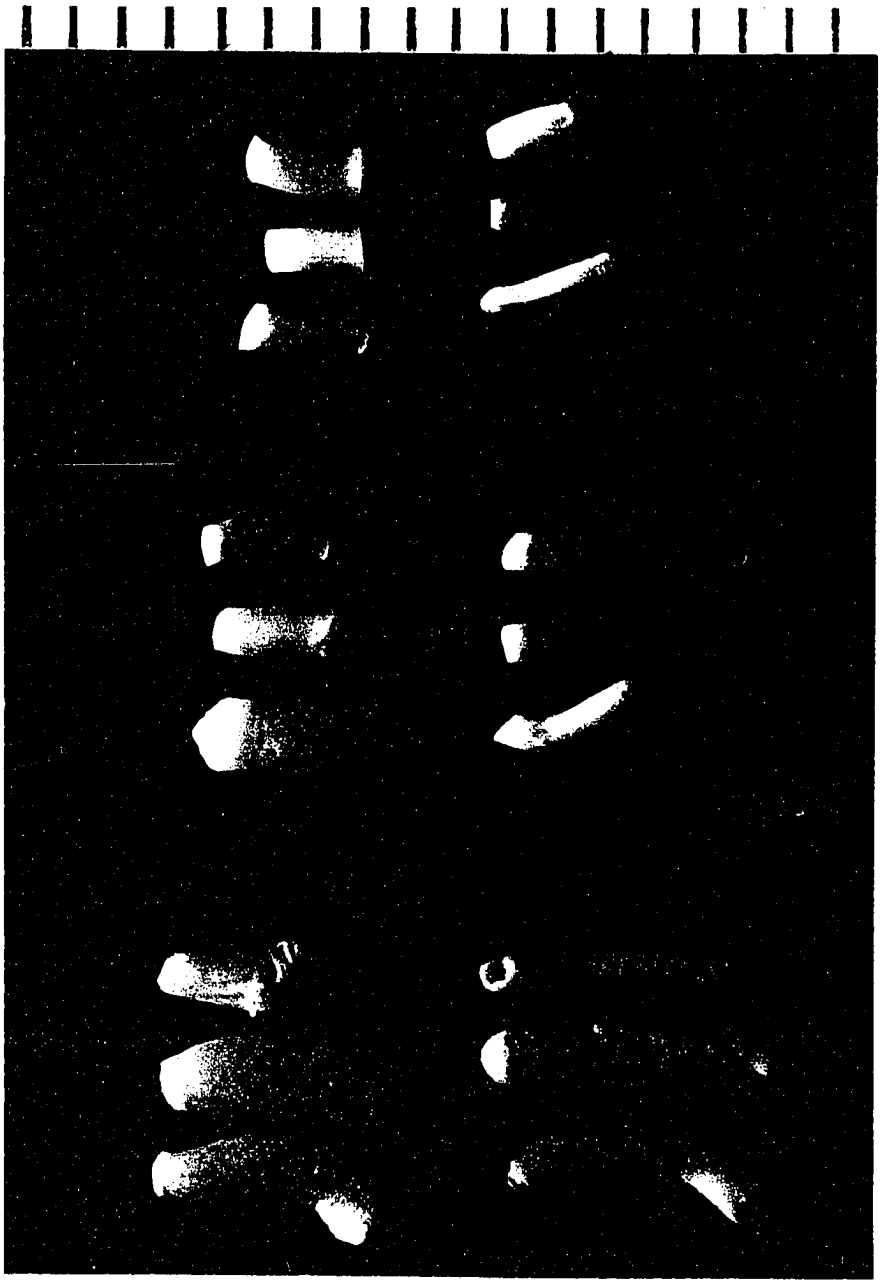
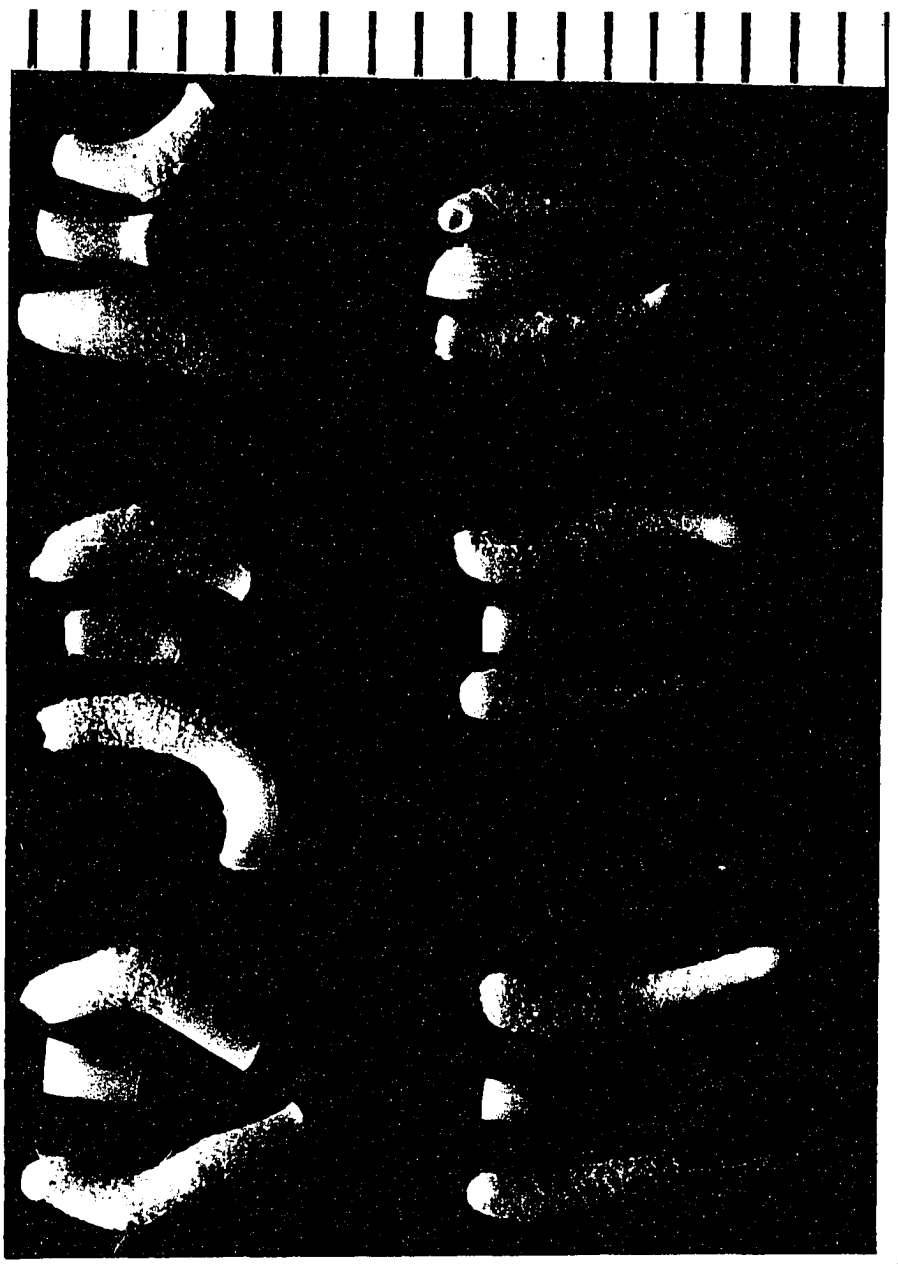


Figure 8. Growth *in vitro* of the division (right column) and enlargement (left column) regions of maize roots at three pH levels with three carbon sources. Top to bottom (by groups of three), pH 5.5, 6.0 and 6.5; within groups, top to bottom, 2% glucose, 2% glucose-1-PO_4, 2% sucrose.



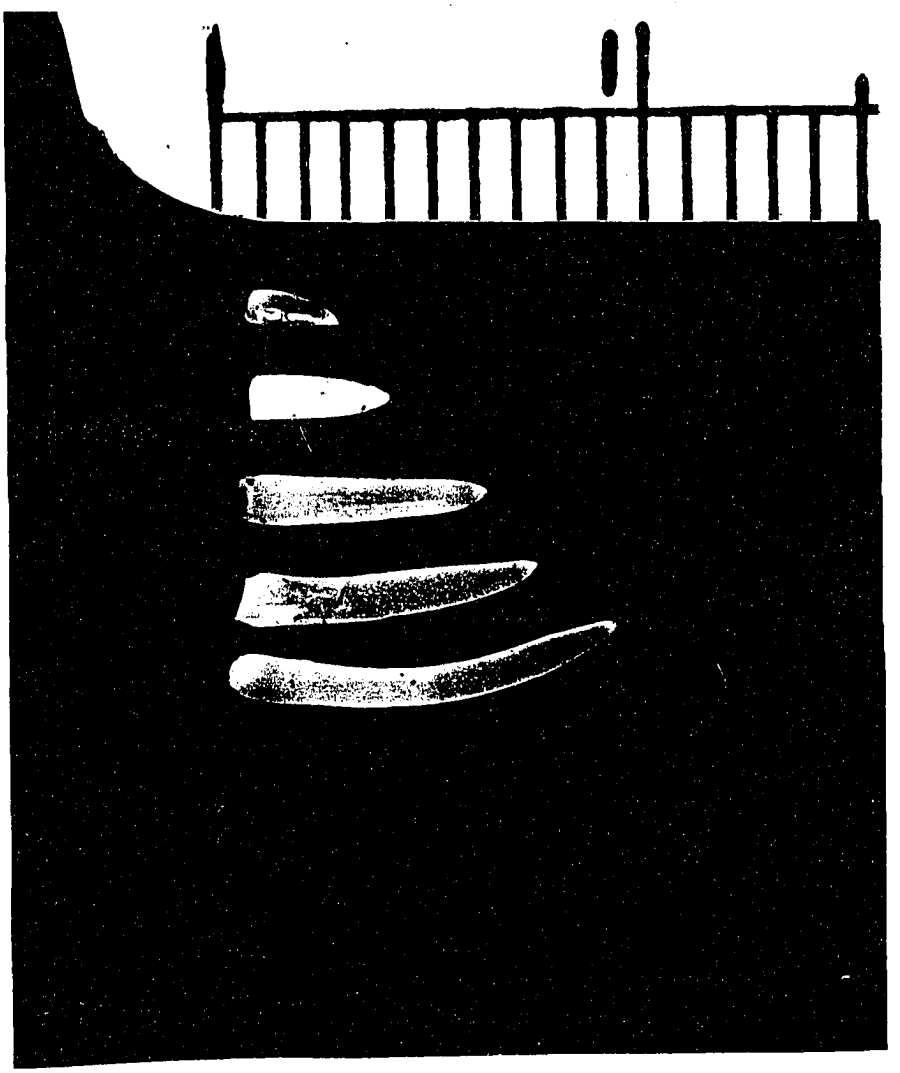


Figure 9. The growth in vitro of the division zone (mm. 1 and 2) of primary maize roots grown in nutrient solution at pH 5.0 with different carbon sources. From top to bottom, length in mm. at the end of 18 hours of the check, glucose, glucose-1-PO_4 and sucrose. At the bottom, a section grown in sucrose at pH 6.5.

RESPIRATORY REACTIONS

Aerobic Respiration

The gas measurements were estimated by placing 50, 2 mm. cuts from each growth zone of germinating seedlings in a Warburg flask. The sample volume was adjusted to 3.00 ml. In the flask for O_2 uptake determinations, 2.6 ml. phosphate buffer, pH 6.0, was added, and 0.20 ml. 20 percent KOH was added in the center wall, allowing 0.20 ml. for tissue volume. In the flask for aerobic CO_2 evolution determinations, the KOH was omitted, adding 2.8 ml. phosphate buffer and allowing 0.20 ml. for tissue volume.

The CO_2 evolution was measured by the Warburg direct method. The correction for bound CO_2 calculated in one experiment was found to be small, and the uncorrected $^{Q}CO_2$ was considered adequate for our comparisons. The results of these determinations were expressed in mu-l taken up per mg dry weight of tissue per hour, $^{Q}O_2$ and $^{Q}CO_2$ and in mu-l taken up per mg tissue nitrogen per hour, $^{Q}O_2(N)$ and $^{Q}CO_2(N)$. The total nitrogen of each individual sample was estimated following the procedure previously described.

Endogenous respiration

Stabilization periods after cutting of 30 minutes, 1 hour and 2 hours were allowed for the material in each set of flasks before closing the manometer stopcock. Each experiment was repeated three times. Two kinds of information were derived from these determinations. First, the changes in respiration rates after different intervals, and second, the reproducibility of rates of respiration found in each growth zone after a specific stabilization interval as measured by the standard error of the mean at the five percent probability level. These data are shown in Table 7.

The rate of O_2 absorption decreased consistently with time after cutting. After a two hour depletion period the $Q_{O_2}(N)$ dropped 31 percent in the division zone, 21 percent in the enlargement zone and 22 percent in the differentiation zone. This trend suggests that the endogenous rate of respiration in the meristematic system of primary maize roots depends largely on a steady supply of substrate from the seed.

After a 30 minute stabilization period the ratio between CO_2 produced and O_2 absorbed (respiratory quotient) in the differentiation zone was found to be 1.02. Further stabilization periods did not alter significantly the respiratory

Table 7. Influence of time after cutting on the endogenous rate of oxygen consumption (Q_{O_2}/mg dry wt., Q_{O_2} mg total nitrogen), aerobic CO_2 evolution (Q_{CO_2}/mg dry wt.,) (Q_{CO_2} mg total nitrogen) and respiratory quotients of meristematic maize root segments at 30 ± 0.25° C.

Region	Time	$\frac{Q_{O_2}}{\text{dry wt.}}$	$\frac{Q_{O_2}}{\text{total N}}$	$\frac{Q_{CO_2}}{\text{dry wt.}}$	$\frac{Q_{CO_2}}{\text{total N}}$	Resp. quot.
Division mm. 1 and 2	30 min.	7.1±0.9	84±13	6.3±1.0	71±17	0.88
	1 hr.	6.3±1.3	73±17	5.8±1.5	66±15	0.94
	2 hrs.	5.1±1.6	58±21	5.3±1.8	61±19	1.05
Enlargement mm. 3 and 4	30 min.	7.6±1.0	106±12	7.1±1.3	100±12	0.94
	1 hr.	6.9±1.5	98±16	6.8±1.1	94±14	0.98
	2 hrs.	4.2±2.1	84±27	4.9±1.3	87±17	1.15
Differentiation mm. 5 and 6	30 min.	6.9±0.7	78±8	7.1±0.6	76±12	1.02
	1 hr.	6.2±1.0	75±10	6.1±1.0	72±14	0.99
	2 hrs.	5.6±1.2	61±12	5.8±1.1	60±13	1.03

quotient in this region which remained very close to 1.00. Respiratory quotients around 1.00 indicate complete combustion of a carbohydrate molecule. The respiratory quotients found in the fifth and sixth millimeter section suggest complete oxidation of carbohydrate molecules and/or compounds with similar oxygen/carbon ratios. The fifth and sixth millimeter sections have shown the largest concentration of carbohydrates, (cf. Table 2). The respiratory mechanisms operating in these sections could be adjusted primarily to the breakdown of such substrate because of availability.

In the enlargement zone, a 30 minute depletion period yielded a respiratory quotient of 0.94, suggesting incomplete oxidation of a carbohydrate substrate, probably with the production of other oxygen respiratory intermediates still oxidizable, rather than CO_2 and water. After a one hour depletion period, the respiratory quotient increased to 0.98 and after a two hour period it increased to 1.15; this excess of CO_2 evolution over O_2 consumption provides indirect evidence of respiratory intermediate accumulation, (aerobic glycolysis), related to prolonged depletion periods, thus yielding larger proportions of CO_2. A comparison of the $Q_{O_2}(N)$ ("unit of respiring protoplasm") within the same radicles, indicated that the enlargement zone (third and

fourth millimeter segment), showed the largest $^{Q}O_2(N)$ and $^{Q}CO_2(N)$, amounting to an average increase of 20 percent over the other two segments. Such respiratory activity could be related to a relatively large endogenous supply of substrate and adequate provision of minerals, as found especially in the third millimeter section from the root apex (cf. Table 2).

In the division zone, (first and second millimeter), a 30 minute depletion period brought about a respiratory quotient of 0.88, suggesting oxidation of compounds with low oxygen/carbon ratio and hydrogen content larger than in carbohydrates, (e g. proteins or fats). The oxidative breakdown of these compounds requires more than one molecule of oxygen for each molecule of CO_2 produced, and respiratory quotients smaller than 1.00 would result. The observed respiratory behavior in the first 2 mm. segment may have been regulated by the nature of the available substrate. In these zones there was found to be a relatively large concentration of protein nitrogen and a low concentration of carbohydrates, (cf. Table 2). One and two hour depletion periods increased the respiratory quotient to 0.94 and 1.05 respectively, indicating again some changes in the concentration of available respiratory intermediates.

The extent to which these results could be reproduced was estimated by comparing standard errors. If these determinations were repeated under similar experimental conditions with similar material, 95 percent of the times the new endogenous respiration rate mean would fall within the range found in each growth region. This comparison showed that the enlargement zone had relatively the largest mean discrepancy at any stabilization period, being more accentuated as these became longer. The division zone showed standard errors more clustered around the mean, but also the discrepancies were larger as the depletion periods became longer. The differentiation zone showed the smallest standard error. These variations in the division and enlargement zones probably indicate larger susceptibility even to slight difference in handling than the differentiation region.

Exogenous respiration

The effect of the addition of respiratory substrate was estimated in the meristematic regions of primary maize roots. After an equilibrium period of two hours, 0.5 ml. of a 2 percent sucrose in solution and in nutrient media was added. This was followed by a new induction period of one hour, after which the gas exchange was measured again.

Runs on respiratory quotients were made at 4, 7 and 10 hours. In the tenth hour the rates reached a peak not exceeded during extended periods. The changes in O_2 uptake and CO_2 evolution are shown in Tables 8 and 9.

In the division zone, sucrose in nutrient solution showed larger effects on the endogenous $Q_{O_2}(N)$ than sucrose alone, amounting to an average increase of 30 percent. The addition of substrate stimulated CO_2 evolution over O_2 uptake and therefore the respiratory quotient increased. The sections suspended in sucrose showed an increase in respiratory quotient from 0.88 (endogenous) to 1.10 (exogenous), while sections suspended in sucrose and nutrient solution showed an increase in respiratory quotient from 0.88 (endogenous) to 1.03 (exogenous). These results indicated some shifts from protein to carbohydrate utilization.

In the presence of external substrate, the enlargement zone showed larger O_2 consumption than the division zone. In sucrose alone, the O_2 uptake was found to be around 20 percent higher than that of the division zone; in sucrose and nutrient solution around 13 percent higher than that of the division zone. This somewhat large O_2 uptake of the enlargement zone was probably related to larger absorption of sucrose which may have been induced

Table 8. The effect of added sucrose substrate on the oxygen consumption (Q_{O_2} per mg dry wt., Q_{O_2} per mg total nitrogen), and aerobic CO_2 evolution (Q_{CO_2} per mg dry wt., Q_{CO_2} per mg total nitrogen) of meristematic maize root segments incubated at 30 ± 0.25° C.

Region	Hour	Q_{O_2} per mg dry wt.	Q_{O_2} per mg total N	Q_{CO_2} per mg dry wt.	Q_{CO_2} per mg total N	Resp. quot.
Division mm. 1 and 2	4 - 5	10.7±1.0	115±10	11.1±.90	98±10	1.04
	7 - 8	12.4±1.6	127±17	13.5±1.8	138±16	1.09
	10 - 11	12.0±1.6	132±18	13.2±2.0	140±20	1.10
Enlargement mm. 3 and 4	4 - 5	12.7±1.3	152±15	13.9±1.4	162±14	1.09
	7 - 8	14.3±2.8	196±38	16.3±2.5	222±16	1.14
	10 - 11	13.9±1.9	182±25	15.9±2.1	206±17	1.14
Differentiation mm. 5 and 6	4 - 5	6.7±1.2	85±12	7.0±1.0	86±14	1.03
	7 - 8	7.6±2.5	89±24	8.0±1.8	92±17	1.05
	10 - 11	7.5±3.4	79±24	8.0±3.1	77±22	1.07

Table 9. The effect of added sucrose plus nutrient solution on the oxygen consumption (Q_{O_2} per mg dry wt. and per mg total N), aerobic CO_2 evolution (Q_{CO_2} per mg dry wt. and per mg total N) of meristematic maize root segments incubated at 30 ± 0.25° C.

Region	Hour	Q_{O_2}		Q_{CO_2}		Resp. quot.
		per mg dry wt.	per mg total N	per mg dry wt.	per mg total N	
Division mm. 1 and 2	4 - 5	10.5±1.6	110±7	10.3±1.0	106±7	0.98
	7 - 8	11.0±1.8	150±19	11.1±2.0	152±10	1.01
	10 - 11	13.5±2.0	172±21	13.9±1.1	177±14	1.03
Enlargement mm. 3 and 4	4 - 5	14.3±1.6	182±21	15.0±1.5	189±13	1.05
	7 - 8	14.9±0.7	202±9	16.1±1.0	218±17	1.08
	10 - 11	14.5±1.5	210±19	15.9±2.0	229±20	1.10
Differentiation mm. 5 and 6	4 - 5	8.3±.4	72±5	8.6±.6	75±10	1.03
	7 - 8	8.4±1.0	68±24	8.3±.9	66±15	0.99
	10 - 11	8.1±1.3	65±25	8.3±1.6	64±17	1.02

by more efficient absorbing mechanisms operating in this region. The respiratory quotient did not vary from the endogenous value (cf. Table 7) and showed slight tendencies toward reduced aerobic glycolysis.

In the fifth and sixth millimeter the increase in the rates of gas exchange in the presence of substrate was found to be non-significant, suggesting a rather limited metabolic capacity and not so much starvation. Similarly, the respiratory quotients remained almost unchanged as compared to the endogenous respiratory quotients.

Respiratory Inhibitors

A preliminary investigation of the nature of the respiratory systems in maize root tip meristems was based on the assumption that any qualitative difference between the mechanisms operating in the division and enlargement zones could be indirectly obtained by comparing the effects of added respiratory inhibitors on the gas exchange capacity of these zones. Chief attention was given to cyanide which has been suggested to be a poison for the metal containing oxidase systems (8) and to iodoacetate because of a postulated relationship between an iodoacetate sensitive fraction

of respiration and elongation (10).

Effect of cyanide

The partial cyanide inhibition of endogenous Q_{O_2} observed in the division and enlargement zone (Table 10) suggests the existence of one or more cyanide stable respiration systems and one or more cyanide sensitive respiration systems. In the presence of sucrose, the Q_{O_2} of either zone increased and also the percentage inhibition, since the amount of cyanide stable respiration was found to remain constant, particularly at the higher cyanide concentrations. This behavior suggests that the addition of sucrose was not shared by the two systems, and that the cyanide stable respiration system seemed to be separated from the cyanide sensitive system.

At any molar concentration the division zone showed a lower percentage inhibition than the enlargement zone. Such response was probably related to the nature of the predominating substrate. The division zone has been found to be rich in proteins and low in carbohydrates (cf. Table 2) and the opposite held for the enlargement zone. Earlier findings (8) have indicated that cyanide sensitive systems appear to use carbohydrates and similar substrates predomi-

Table 10. The effect of cyanide concentration on the endogenous and exogenous O_2 uptake ($^{Q}O_2$/mg dry wt.) of primary maize root meristematic sections incubated at 30 ± 0.25° C.

Cyanide molar conc.	Region	Endogenous resp. $^{Q}O_2$/mg dry wt. 1 hour	Endogenous resp. $^{Q}O_2$/mg dry wt. 3 hours	Cyanide sensitivity*	Exogenous $^{Q}O_2$/mg dry wt. 1 hour	Exogenous $^{Q}O_2$/mg dry wt. 3 h
0	Division	6.3±1.0	4.2±0.8		12.0±2.0	12.
	Enlargement	7.5±1.0	4.3±1.2		14.5±1.8	14.
1×10^{-6}	Division	5.2±1.3	3.4±0.6	3.	12.5±2.3	12.
	Enlargement	7.1±1.2	4.2±1.5	4.	14.2±1.8	12.
1×10^{-5}	Division				10.8±2.2	8.
	Enlargement				14.9±2.1	5.
1×10^{-4}	Division	6.6±1.2	3.8±.3	42.	12.4±1.3	4.
	Enlargement	7.6±1.0	3.2±.8	58.	13.8±0.9	2.
1×10^{-3}	Division				12.0±1.9	2.
	Enlargement				14.3±2.1	1.
1×10^{-2}	Division	6.6±1.5	1.7±0.4	74.	12.1±1.7	1.
	Enlargement	7.0±0.9	0.0	100.	13.5±2.7	

*Cyanide sensitivity at the end of 3 hours = $\frac{\text{Original resp. rate} - \text{Cyanide resp. rate}}{\text{Original resp. rate}} \times 100$

ranide concentration on the endogenous
uptake ($^{Q}O_2$/mg dry wt.) of primary
stematic sections incubated at 30 ±

Endogenous resp. $^{Q}O_2$/mg dry wt. 1 hour	3 hours	Cyanide sensitivity*	Exogenous resp. $^{Q}O_2$/mg dry wt. 1 hour	3 hours	Cyanide sensitivity*
5.3±1.0	4.2±0.8		12.0±2.0	12.30±1.3	
7.5±1.0	4.3±1.2		14.5±1.8	14.10±1.6	
5.2±1.3	3.4±0.6	3.	12.5±2.3	12.4±1.6	0.
7.1±1.2	4.2±1.5	4.	14.2±1.8	12.0±1.4	15.
			10.8±2.2	8.2±1.2	24.
			14.9±2.1	5.9±1.5	60.
5.6±1.2	3.8±.3	42.	12.4±1.3	4.0±1.0	67.
7.6±1.0	3.2±.8	58.	13.8±0.9	2.9±1.5	79.
			12.0±1.9	2.3±0.4	81.
			14.3±2.1	1.4±0.2	98.
5.6±1.5	1.7±0.4	74.	12.1±1.7	1.8±0.4	85.
7.0±0.9	0.0	100.	13.5±2.7	0.0	100

$$\frac{\text{al resp. rate} - \text{Cyanide resp. rate}}{\text{Original resp. rate}} \times 100$$

nantly, while stable systems tend to oxidize protein or fats, and that most variations in O_2 uptake occur within the cyanide sensitive systems alone. From this differential cyanide susceptibility between the division and enlargement zones it became apparent that the cyanide sensitive system operating in the enlargement zone was very active and responded more readily to variations in some of the factors which affect the Q_{O_2} (e g. sucrose addition), while the cyanide stable respiration system operating in the division zone accounted for a small amount of oxygen consumption.

The in vitro effects of cyanide were followed on the apical 2 mm. segments suspended in aerated nutrient solution plus 1 x 10^{-9}M/l indoleacetic acid during 18 hours at 30° C. The observed growth response and the induced chemical changes are given in Table 11.

Qualitatively, growth appeared to be more sensitive to cyanide than respiration, particularly at the higher cyanide concentrations. Tissues exposed to the action of 1 x 10^{-2}M/l and 1 x 10^{-3}M/l of cyanide were killed, (cf. Fig. 10), and although sections treated with 1 x 10^{-4}M/l showed a gain in section length, there was a loss of green material equivalent to 85 percent of the check. It seems probable

therefore, that cyanide interfered either with the formation of essential growth intermediates or with the utilization of energy derived from some of the aerobic respiration processes.

Effect of iodoacetate

A representative sample of the zones of division, (mm. 1 and 2), and enlargements, (mm. 3 and 4), was suspended in aerated nutrient solution plus 1 x 10^{-9}M/l indoleacetic acid. After an equilibrium period of 30 minutes the manometer stopcock was closed and the exogenous respiratory quotient was estimated in the following hour. Iodoacetic acid in concentrations ranging from 5 x 10^{-6} to 1 x 10^{-8}M/l at 0.5 was added at the end of the second hour. This was followed by a new induction period of one hour, after which the gas exchange was measured again. These data are shown in Table 12.

The respiratory quotient decreased. The $^{Q}CO_2$ decreased rapidly during the first three hours after the addition of the inhibitor. It then leveled off to a minimum volume not exceeded during an extended period of time, which indicated maximum inhibition. In the division zone the strongest concentration reduced aerobic CO_2 evolution to 70 percent

Table 11. The *in vitro* effect of cyanide on the apical 2 mm., meristematic portion of maize root grown in nutrient solution plus 1 x 10^{-9} M/l indoleacetic acid and incubated for 18 hours at 30° ± 25° C.

Cyanide M/l	Length in mm.	Diameter in mm.	Gamma fresh wt.	Total sugars, % grn. wt.	Soluble N, % grn. wt.	Alcohol insoluble matter.
			At beginning of experiment:			
Check	2.0	0.8	1750	0.75	0.20	260
			At end of 18 hours:			
Check	7.5	1.0	5350	1.86	0.37	390
$1x10^{-5}$	5.1	1.2	4280	3.22	0.65	160
$1x10^{-4}$	4.2	1.0	270	0.10	0.75	68
$1x10^{-3}$	3.1	0.7	183	0.03	0.07	21
$1x10^{-2}$	2.0	0.5	175	0.05	0.10	18

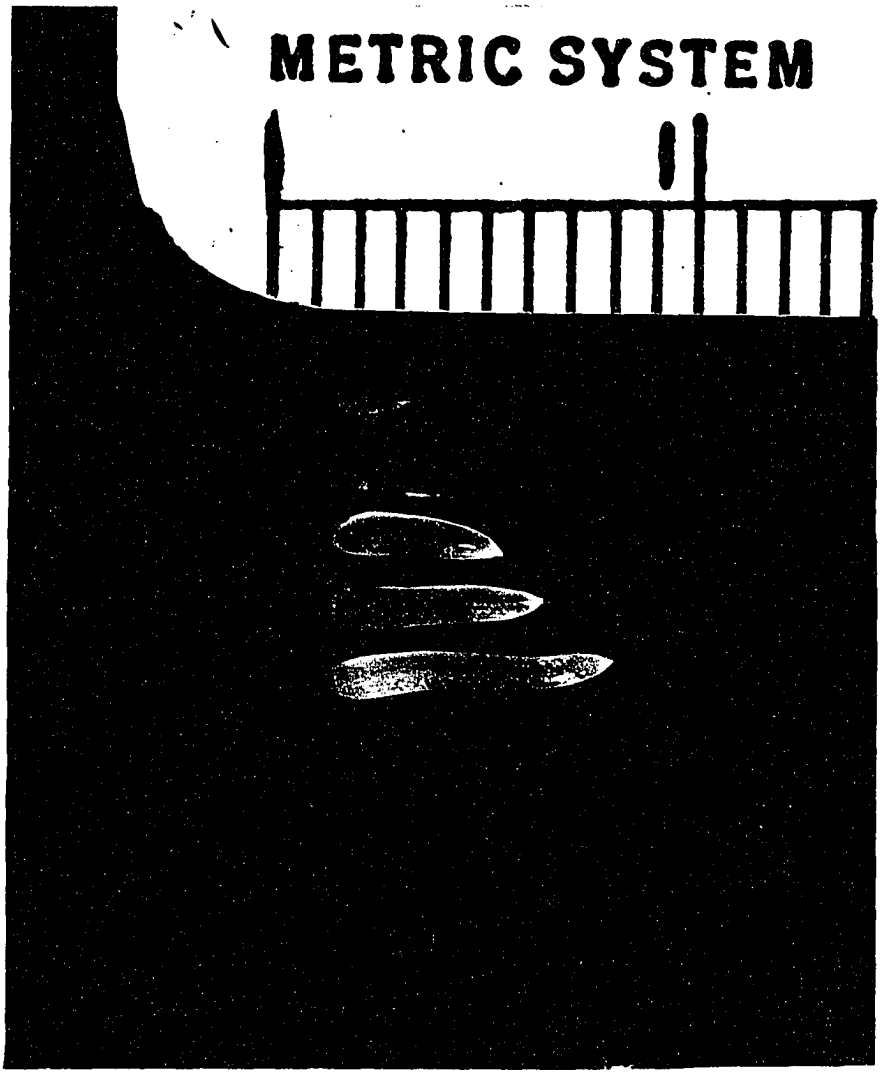


Figure 10. The effect of cyanide on the development of the division zone (mm. 1 and 2) of primary maize roots grown in vitro in nutrient solution. From top to bottom, length in mm. at the end of 18 hours of the sections grown in 1×10^{-2}, 1×10^{-3}, 1×10^{-4}, 1×10^{-5} M/l and check.

of the original exogenous volume, while the enlargement zone showed more sensitivity to iodoacetate. In the range 1×10^{-8} to 1×10^{-6} M/l, the evolution of CO_2 varied from 73 to 15 percent of the check, respectively.

This differential iodoacetate sensitivity was probably related to the concentration and type of metabolite substrate in each growth zone. The probable nature of iodoacetate action has been shown to be due to its combination with -SH radicals and recently it has become known that proteins containing sulphydryl groups are essential components of a large number of enzyme complexes. The wide range of such enzymes leaves a relatively large number of respiration and growth processes on which iodoacetic acid may act. The tendencies toward reduced aerobic glycolysis suggest that the principal site of inhibition may be the triose-phosphate dehydrogenase system, shown previously in bean stem slices (44) and yeast (16).

The effects of similar iodoacetic acid concentrations were measured on the growth and chemical composition of the apical 2 mm. section grown in nutrient solution plus 1×10^{-9} M/l indoleacetic acid. Tissues exposed to 5×10^{-6} M/l were killed and 5×10^{-7} and 5×10^{-8} M/l produced distinct deformations such as partial ruptures and bending (Fig. 11). Northen (36) suggested that protein dissociation leads to increases in

Table 12. The influence of monoiodoacetic acid on the respiratory quotient of primary maize root meristematic sections incubated at 30 ± 0.25° C.

Iodoacetate M/l	Hours	Division(mm.1&2) Q_{O_2}/ mg dry wt.	Resp. Quot.	% Inh.	Enlargement(mm.1&2) Q_{O_2}/ mg dry wt.	Resp. Quot.	% Inh.
1×10^{-8}	1 - 2*	10.5	1.03		12.5	1.00	
	3 - 4	11.0	1.03	0	15.0	0.78	22
	4 - 5	15.5	1.01	2	14.2	0.75	22
	11 - 12	16.0	1.01	2	16.9	0.73	27
5×10^{-8}	1 - 2*	13.3	0.99		11.4	1.02	
	3 - 4	14.6	0.90	9	18.1	0.69	32
	4 - 5	19.4	0.88	11	20.1	0.49	52
	11 - 12	18.3	0.88	12	13.4	0.43	58
1×10^{-7}	1 - 2*	11.4	1.02		11.8	1.08	
	3 - 4	16.0	0.91	11	16.4	0.53	51
	4 - 5	18.9	0.91	11	15.1	0.45	59
	11 - 12	11.4	0.89	13	14.5	0.44	58
5×10^{-7}	1 - 2*	11.7	1.07		12.1	1.10	
	3 - 4	11.7	0.97	9	17.9	0.72	35
	4 - 5	18.1	0.95	12	19.0	0.63	52
	11 - 12	17.7	0.93	13	11.6	0.40	64
1×10^{-6}	1 - 2*	10.9	1.00		12.1	1.00	
	3 - 4	17.4	0.90	9	13.6	0.45	55
	4 - 5	20.2	0.87	13	11.7	0.29	71
	11 - 12	17.0	0.85	15	9.7	0.15	85
5×10^{-6}	1 - 2*	12.7	0.98				
	3 - 4	14.0	0.74	25			
	4 - 5	14.3	0.72	27			
	11 - 12	13.6	0.69	30			

*Inhibitor added at the end of second hour.

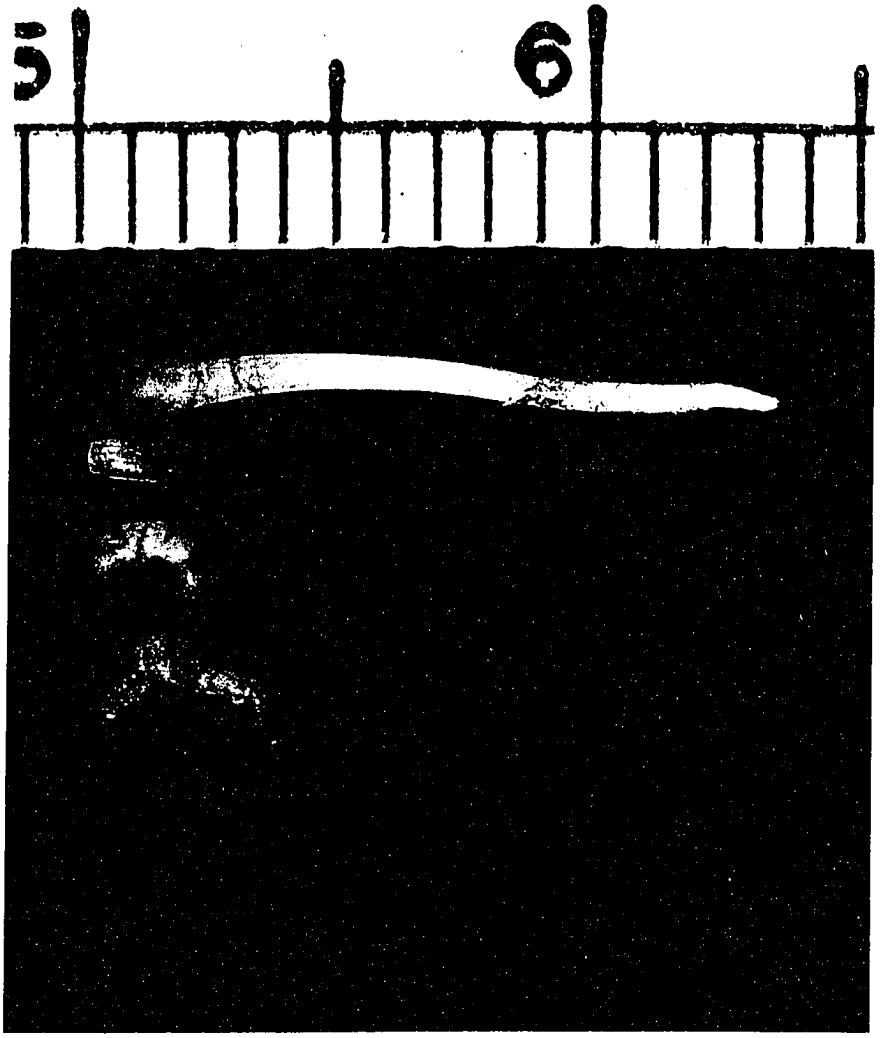


Figure 11. The effect of monoiodoacetic acid on the development *in vitro* of the division zone (mm. 1 and 2) of primary maize roots grown in nutrient solution. From top to bottom, appearance at the end of 24 hours of the check, 5×10^{-6}, 5×10^{-7} and 5×10^{-8} M/l.

peptides with -SH groups. The presence in the first and second millimeter of large proportions of sulphydryl enzymes which are known to catalyze dehydrogenation processes (37), is postulated. A complete immobilization of the liberated -SH groups essential for dehydrogenase and carbohydrase action may explain the observed inhibition of growth by iodoacetic acid.

HYDRATION

The hydration of a tissue may be estimated quantitatively from its relative proportions of water and dry matter. We have used the ratio

$$\frac{\text{Green weight} - \text{dry weight}}{\text{Dry weight}} = \text{Hydration ratio}$$

as an index of this change. The dry weight percentages were determined for the first five 1 mm. regions in radicles grown at 25° C. (Table 2, Fig. 12). The percentage of dry matter decreased nearly three times from the first millimeter to the fifth millimeter. Accordingly, the hydration ratios increased from 4.3 to 14.6 between the first and the fifth millimeter regions. Since cells located in the central cylinder contain larger than average percentages of dry matter, still larger amounts of water may be expected in cortical cells located in the outer shell.

At any radicle level the extent of hydration probably depends upon a balance between external factors (e g. availability of water and temperature) and internal factors (e g. auxin and food supply). An attempt was made to estimate the separate contribution to vacuolation given by each of these factors. The influence of water availability and temperature

on water uptake was measured in the following manner. Two representative samples of the first five 1 mm. sections were weighed in minimum time after cutting, then suspended in aerated distilled water during one hour at 5° and 30° C. respectively. The samples were blotted and fresh and dry weight estimated subsequently. Any departure from the original fresh weight was assumed to be related in part to the elasticity, but particularly to the plasticity of the cell walls. The data of two separate experiments are shown in Table 13.

Dry weights were determined at the end of the experiments and reflect the greater respiration losses and possibly greater leaching at the higher temperature (cf. Tables 2 and 13). For these reasons the apparently slightly higher hydration obtained at the higher temperature cannot be considered significant. At the lower temperature the estimated hydration was of the order of 2 percent, with very little difference between the sections. Simple swelling by water absorption had very little effect on the hydration of these meristematic tissues.

The interrelationships between sugar, auxin and hydration were estimated in 2 mm. sections of the regions of division and enlargement. One set of samples was used

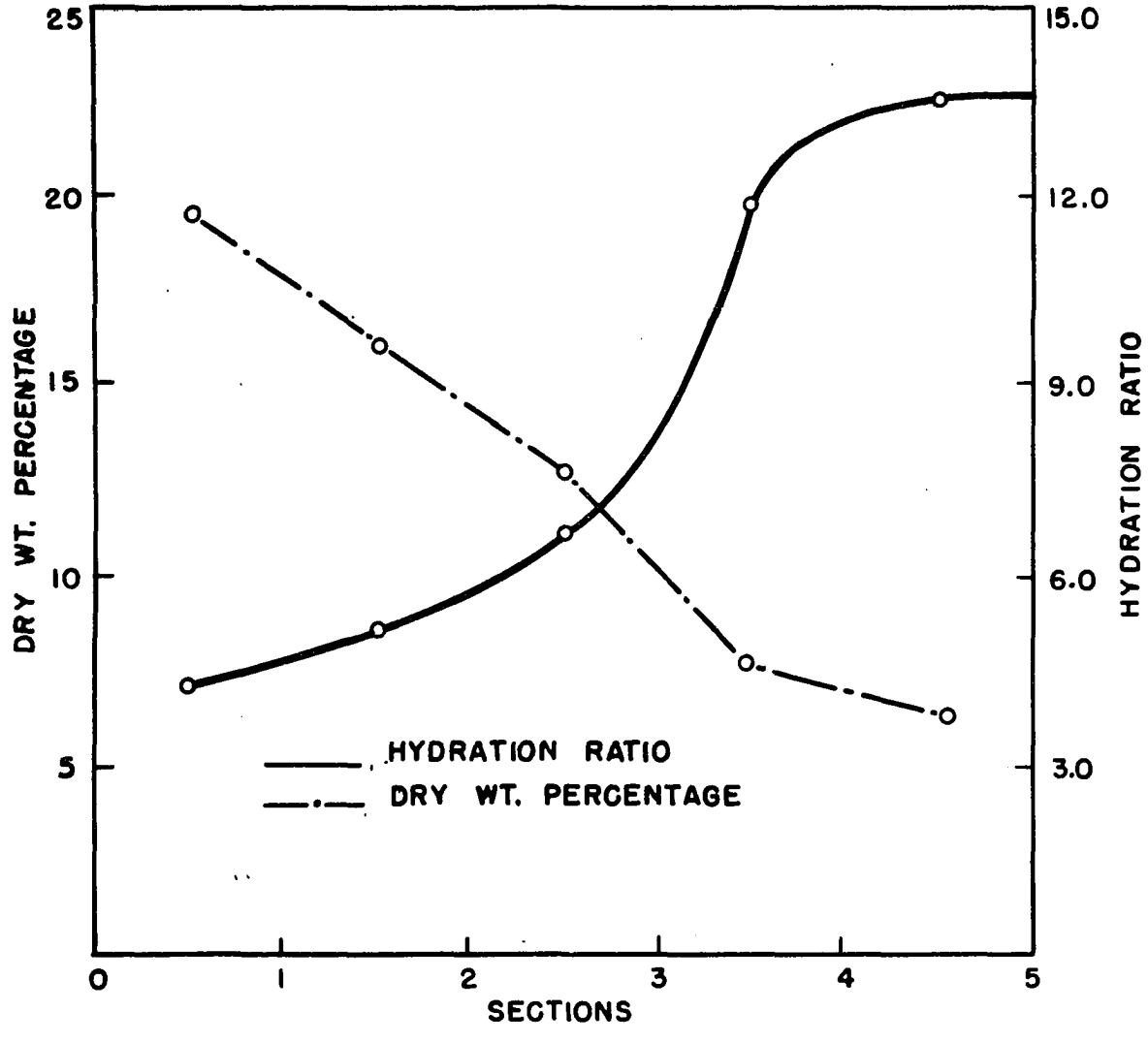


Figure 12. Dry weight percentage and hydration ratio of consecutive 1 mm. segments of primary maize root tips grown at 25° C.

Table 13. The influence of water availability and temperature on water absorption by the first five 1 mm. meristematic regions of primary maize roots.

Mm. from tip	Temp. °C.	Gamma grn. weight Before soak	Gamma grn. weight After soak	Gamma dry wt.	Dry wt. % Before	Dry wt. % After
1	5	665	684	126	19.0	18.5
	30	643	662	117	18.2	17.1
2	5	1122	1170	181	16.1	15.5
	30	1110	1200	168	15.1	14.0
3	5	1235	1258	151	12.3	12.0
	30	1210	1306	144	11.9	11.0
4	5	1380	1410	104	7.6	7.4
	30	1370	1425	100	7.3	7.0
5	5	1600	1640	100	6.2	6.1
	30	1560	1590	92	5.9	5.7

Table 14. The influence of sucrose and auxin on water absorption by the meristematic regions of primary maize roots incubated at 30° C.

Substrate	Region	Gamma fresh wt.	Gamma dry wt.	Dry wt. %	Q_{O_2}(N)	Hydration ratio*
		At beginning of experiment:				
Check (water)	Division	1770	300	16.9	80±15	4.9
	Enlargement	2600	260	10.0	705±10	9.0
		At end of second respiration test:				
Check (water)	Division	1800	280	15.5	54±25	5.5
	Enlargement	2760	245	8.9	78±18	10.3
Sucrose	Division	2020	266	13.2	110±15	6.6
	Enlargement	2860	190	6.7	159±16	13.9
$1x10^{-9}$M/l I.A.A.	Division	2145	237	11.1	130±12	8.1
	Enlargement	3222	162	5.0	224±20	17.3
Sucrose + I.A.A.	Division	2362	274	11.6	116±10	7.7
	Enlargement	3620	163	4.5	185±15	21.2

*Hydration ratio = $\frac{100 - \text{percent dry weight}}{\text{percent dry weight}}$

to determine the original green and dry weights of the sections. A second set was cut, weighed in the minimum time, transferred to Warburg flasks, equilibriated in water for 30 minutes, and their O_2 uptake measured over a one hour period. One-half milliliter of 2 percent sucrose, 1 x 10^{-9} indoleacetic acid, or 2 percent sucrose plus indoleacetic acid was then tipped into the flasks, and O_2 consumption redetermined after a second equilibriation. Fresh and dry weights and nitrogen content of the experimental material were then estimated. The data are shown in Table 14.

The hydration ratio in the division zone suspended in sucrose alone increased 35 percent, while in the enlargement zone it went up 54 percent. Indoleacetic alone increased the hydration ratio 65 percent in the division zone and 92 percent in the enlargement zone. Finally, in the division section suspended in sucrose plus indoleacetic acid the ratio increased 57 percent and in the enlargement section it increased 135 percent. The $^{Q}O_2(N)$ increased more than two times in the division zone, while in the enlargement zone suspended in indoleacetic acid the $^{Q}O_2(N)$ increased nearly three times, and enlargement sections suspended in sucrose and indoleacetic acid it increased two times.

In the presence of auxin and/or sucrose the meristematic regions became metabolically more active, as shown by larger rates of O_2 consumption, and hydration proceeded rapidly. During the time of the experiment, a total of three hours, both sections may be assumed to have reached an older average physiological age. They still maintained their relative positions, however, with hydration in the 3 - 4 mm. section about twice that in the 1 - 2 mm. region.

The reactions shown here could be assigned either to active water absorption dependent upon respiratory energy, or to chemical reactions in cell wall increase. A final choice of reactions is not possible with the data available. Since all of the cells were turgid, however, indicating water absorption by diffusion, and since the combination of auxin and sucrose was appreciably more effective in hydration, although not in respiration, than either alone, the writer feels that a theory of cell wall growth, possibly accompanied by protoplasmic changes, is the most plausible explanation. We visualize some such change as cellulose micelles in the wall being let out by protoplasmic, synthetic action before cell enlargement and hydration can occur. The final step may then be absorption by diffusion alone.

DISCUSSION

Cell Division and Enlargement

These studies on the growing regions of primary maize roots furnish evidence regarding the specific distribution of growth processes at different distances from the root tip. If we ignore the root cap, the first millimeter of maize roots as we have grown them is rounded and somewhat tapered, so that it is 40 percent smaller than the second millimeter section. The cells of the first section are isodiametric, 11 mu long, high in protein (61 percent of dry wt.) and dry matter (about 20 percent). These measurements have been remarkably constant in as many as 45 different determinations of the same character. Generation time for one complete cell division was 29 hours at 15° and 18 hours at 25° C.

In the second millimeter a rapid change from cell division to cell enlargement occurred. The first quarter of this section was similar to the preceeding region except that cell division was perhaps more rapid at higher temperatures. The maximum rate of cell division, with a generation time of less than 10 hours and possibly as low as 6 hours was

estimated for the second quarter of this region. At the same time the average cell length nearly doubled to give a sharp peak of total growth. Elongation continued and cell division slowed in the third and ceased in the fourth quarter of the second millimeter. Average protein nitrogen per cell in the second millimeter was double that in the first and average cell length was not quite double so that rapid protoplasm synthesis was indicated.

The third millimeter grew by enlargement only at a rate one-third less than the combined effects of cell division and enlargement in the second section. Total nitrogen per unit cell length doubled again over the second section but cell length increased nearly three times, so that protein synthesis began to lag. No direct estimates of vacuolation were made. The high protein contents of the second and third sections suggest no more than a slow vacuolation across these rapidly growing regions. The hydration index (water/dry matter), however, increased from 4.2 in the first millimeter to 5.2 in the second and 6.8 in the third. This increased hydration was accompanied by an accumulation of soluble nitrogen and sugars, compounds which would tend to increase hydration by vacuolation.

The change in the hydration ratio reached a maximum

between the third and fourth millimeters where it jumped from 6.8 to 11.8. At the same time dry matter, protein, ash and most cell constituents except sugar and cellulosic materials decreased on a cell unit basis, indicating rapid vacuolation. Because of the inevitable time lag in any growth measurements, the elongation rates assigned to the third millimeter in Table 1 occurred for the most part in the fourth millimeter. For the same reason the low growth totals shown for the fourth millimeter mean a short period before the fourth millimeter became the fifth, rather than a slow rate. Cell length increase between the fourth and fifth millimeter was the second largest observed. Since no measurable elongation was recorded at any time in the fifth millimeter, the changes in this and succeeding sections are considered to be confined to differentiation reactions, among which the increase of cellulosic materials was notable (Table 3).

We have already indicated that the rates of cell division in the first and second millimeters were increased by temperature changes from 15 to 25° C. Specific division rates for the two temperatures were 0.035 and 0.055 in the first millimeter, and 0.036 and 0.092 in the second over a three hour interval (2). A possible rate of 0.126 was estimated for the

first (distal) part of the second millimeter. The rates of elongation also showed high temperature coefficients, on the order of 3 - 4 for a rise of 10° C. in the third millimeter where no cell division occurred. Such a coefficient indicates that chemical rather than physical reactions are limiting for cell elongation processes (7). This relationship is shown clearly in the data of Tables 13 and 14. Root tip sections held in aerated water showed a very small water absorption. The same sections held in 2 percent sucrose solution absorbed a little more water than the checks, but sections in indoleacetic acid, and especially in indoleacetic acid plus sucrose, increased 50 percent in fresh weight in one hour and their hydration ratio doubled. Cell enlargement would seem to be limited by enzymic reactions dependent upon the presence of auxins. We may postulate that these reactions involve the lengthening of the cellulose micelles of the cell wall in such a way as to allow expansion of the cell by hydrostatic pressure.

Although division of vacuolated cells has been shown to take place in certain meristems (e g. stem tip and pith cells), division of rapidly vacuolating cells was not detected in the meristematic tissues of primary maize roots. This observation points out the occurrence, at specific radicle levels, of

irreversible changes in the nature of the meristematic development. The mechanisms of cell division and enlargement which appeared to be closely related in the distal zones later appeared to have been dissociated, and from the third millimeter on, only cell enlargement with or without protoplasm synthesis took place.

The cytoplasm has been regarded as a cyto-skeleton composed of a three-dimensional lattice of protein fibrils (23). The protein material in the apical two millimeter section was relatively dense, as shown by the small cells with their high protein content and viscosity. With hydration the structure of the proteins in this dense structure may be irreversibly altered, somewhat in the manner shown by Rothen (41) for films of a number of proteins. Thus the action of specific enzymic proteins, or perhaps the general level of metabolic and particularly of structural activity might be reduced by the tendency of protein molecules to unfold when hydrated. Such changes in protein structure might account for the failure of hydrated protoplasm to continue division, but would still not explain the original cause of the increased hydration which seems to be a zonal effect, either physical or chemical.

Chemical Constituents

The future of a developing cell seems to be predetermined by its relative position with respect to the incoming food supply and to unknown zonal effects, rather than by the morphological characteristics of the cell itself. Furthermore, meristematic activity involving protoplasmic synthesis appears entirely dependent upon a continuous supply of food materials from which protoplasm may be constructed. In primary maize roots, phloem and xylem end at some distance behind the zone in which active meristematic growth is in progress. From there on, nutrients may penetrate through the vacuolated tissue intervening, and finally an individual cell may receive this food supply by way of its surface.

Total reducing substances after hydrolysis with invertase showed the expected gradient, being highest nearer the endosperm source and lowest in the apical section. Soluble nitrogen, which might also be considered to be a translocation form did not show the same pattern, but was highest in the third millimeter section and lower both apically and basally from this region. Concentrations here suggested synthesis of proteins in the zones of division and digestion in the

third and later zones. There is evidence (27) that storage proteins are retranslocated only after digestion to simpler water soluble forms and that the various soluble organic materials are more or less interchangeable in their effects of stimulating growth. It may be also that soluble nitrogen accumulation in the enlargement zones resulted from local protein digestion. A sharp decline in soluble nitrogen with the corresponding increase in colloidal nitrogen may be indicative of optimal environmental conditions required for the condensation of simple nitrogenous compounds into proteins and new protoplasm.

Respiration

Respiration measurements on the isolated meristematic regions of primary maize roots led to the differentiation of each growing region in terms of their distinct respiratory activities. The respiration rates found in each zone probably were dependent upon a specific internal balance between the nature of the available respiratory substrate and the amount of protoplasm (respiratory systems) per unit volume of respiring tissue. This balance determined the final respiration rate per unit of respiring protoplasm. Thus, the

respiratory quotient calculated for the fifth and sixth millimeter region which amounted to 1.00 suggested complete combustion of carbohydrate molecules predominantly. These sections showed the largest concentration of carbohydrates. The respiratory quotient of the first and second millimeter was of the order of 0.88, suggesting combustion of compounds with low oxygen/carbon ratio, e g. proteins or fats. Similarly, these zones were characterized by relatively large concentration of protein nitrogen.

Since the energy produced by cellular oxidations cannot be thought of in terms of a pooled fund which is merely apportioned to several endothermic processes according to need, a close relationship between each energy producing respiratory process and the endothermic function which uses the energy thus produced may be postulated. This connection may be due to a specific orientation between the two processes concerned.

In view of the suggestion (44) that auxin stimulates growth by affecting the generation of available chemical energy and/or substrate during the respiratory processes, it may be then that the changes in the meristematic development of primary maize roots, may be brought about by some participation of auxin or auxins on the respiratory reactions

of the living protoplasm. Each partial reaction or formation of an intermediate and its subsequent degradation must have at least a velocity equal to the velocity of the overall reaction of respiration. The rate of formation and removal of each respiratory intermediate during the respiration cycles may be a critical factor since presumably some of these intermediates are utilized as substrate or catalytically. Also, not only the life of an intermediate compound may be short but its concentration also low.

The data of Tables 7 and 8 show endogenous and exogenous respiration of the three isolated development zones represented by millimeters 1 and 2, 3 and 4, and 5 and 6. Endogenous respiration tended to be highest in the middle section, zone of cell enlargement, but differences were surprisingly small and the zone of differentiation was almost as high as either of the others. Exogenous respiration rates in Table 8 show that the relatively high rate of the differentiation zone in Table 7 was due to its high sugar content. Exogenous respiration rates were clearly highest in the zone of enlargement, second in the zone of cell division and a poor third in the third zone. The addition of nutrient salts to the sucrose solution did not change the picture (Table 9).

The inhibiting effects of cyanide and iodoacetic acid

on root respiration are shown in Tables 10 and 12. Exogenous respiration was more sensitive to cyanide than endogenous, and the enlargement zone was more sensitive than the zone of division. Depression reached 100 percent in the enlargement zone. At 10^{-2}M iodoacetate was about five times more inhibiting on the respiration of the enlargement zone, thus suggesting basic differences in the respiration of the division and enlargement regions.

SUMMARY

Growth of the maize roots observed has been limited to the first four millimeters behind the root cap. Growth in the first millimeter involved cell division at a moderate rate with enlargement of the daughter cells to the original cell size but not above. Growth in the second millimeter included both the most rapid cell division and the most rapid elongation, although the division tended to be concentrated in the apical half and the elongation in the proximal half of the section. Growth of the third and fourth millimeters was by cell enlargement only.

Cell division in the maize root tip ceased before hydration became marked and probably did not occur in clearly vacuolated cells. Cell generation time has varied with temperature and position from 8 hours or less to nearly 30. The temperature coefficient (Q_{10}) is of the order of 2.

Cell enlargement has shown a temperature coefficient near 4, indicating that factors limiting the rate of cell enlargement are of the nature of chemical forces and that the overall rate of cell enlargement is limited by the slowest

of the rates of the various chemical reactions involving simultaneous cellulose synthesis and increases in cell wall plasticity. When one or more of these intermediate reactions reaches a critical rate, the whole rate of cell enlargement slows down or ceases.

The chemical determinations of available compounds showed that total reducing substances were highest in the proximal sections. Soluble nitrogen did not show the expected gradient but was highest in the third millimeter section and lower both apically and basally from this region. Protein synthesis in the division zone and local protein digestion in the enlargement zone are indicated.

Endogenous and exogenous respiration rates were clearly highest in the zone of enlargement. The greater susceptibility to cyanide and iodoacetate inhibition in the enlargement zone suggests basic differences in the nature and activity of the respiratory systems operating in these zones.

The temperature coefficients for cell enlargement, the relatively large accumulation of soluble nitrogen, the high respiration rate and the sensitivity to inhibitors, provide several indications that the meristematic regions of primary maize roots undergoing cell enlargement include the most active centers of the root in many metabolic functions.

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